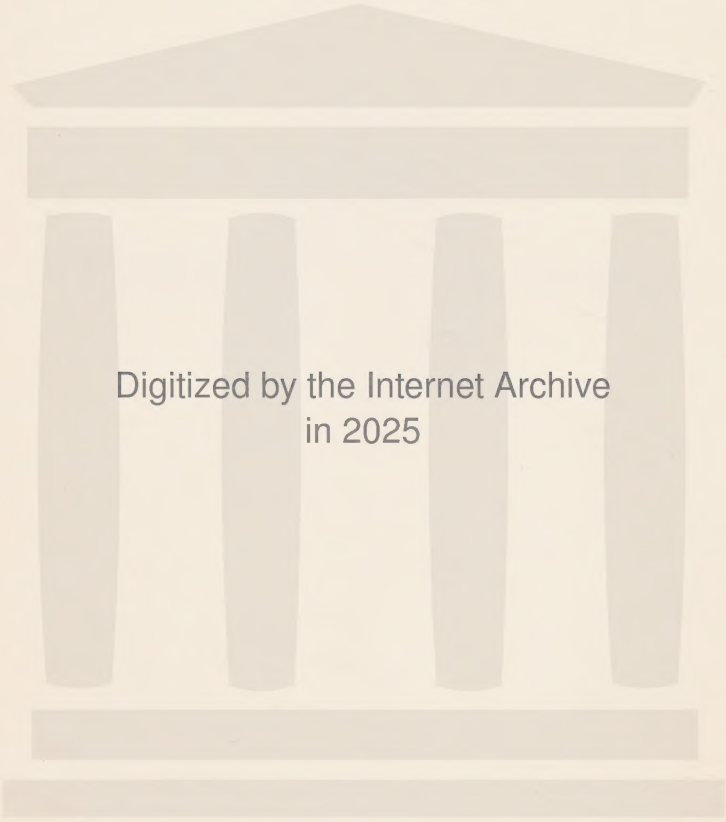
LANE MEDICAL LIBRARY STANFORD
F31 .R72 1927 OLD
Textbook of comparative physiology / by
24503098293

LANE
MEDICAL

LIBRARY
Gift
Dr.Robert Watkins

Robert P. Watkins,
124 Fountain Avenue,
Pacific Grove, California.


Digitized by the Internet Archive
in 2025

https://archive.org/details/textbookofcompar0000char

McGRAW-HILL PUBLICATIONS IN THE ZOÖLOGICAL SCIENCES

A. FRANKLIN SHULL, Consulting Editor

TEXTBOOK OF COMPARATIVE PHYSIOLOGY

McGRAW-HILL PUBLICATIONS IN THE ZOÖLOGICAL SCIENCES

A. Franklin Shull, Consulting Editor

Fernald—
APPLIED ENTOMOLOGY

Mitchell—
GENERAL PHYSIOLOGY

Pearse—
ANIMAL ECOLOGY

Rogers—
COMPARATIVE PHYSIOLOGY

Shull—
HEREDITY

Shull, Larue and Ruthven—
ANIMAL BIOLOGY

Shull, Larue and Ruthven—
LABORATORY DIRECTIONS IN ANIMAL BIOLOGY

Snodgrass—
ANATOMY AND PHYSIOLOGY OF THE HONEYBEE

Van Cleave—
INVERTEBRATE ZOOLOGY

Wieman—
GENERAL ZOÖLOGY

McGRAW-HILL PUBLICATIONS IN THE AGRICULTURAL AND BOTANICAL SCIENCES

Edmund W. Sinnott, Consulting Editor

Adams—
FARM MANAGEMENT

Babcock and Clausen—
GENETICS IN RELATION TO AGRICULTURE

Babcock and Collins—
GENETICS LABORATORY MANUAL

Boyle—
MARKETING OF AGRICULTURAL PRODUCTS

Brown—
COTTON

Carrier—
BEGINNINGS OF AGRICULTURE IN AMERICA

Cruess—
COMMERCIAL FRUIT AND VEGETABLE PRODUCTS

Cruess and Christie—
LABORATORY MANUAL OF FRUIT AND VEGETABLE PRODUCTS

Eames and MacDaniels—
PLANT ANATOMY

Emerson—
SOIL CHARACTERISTICS

Fawcett and Lee—
CITRUS DISEASES

Gardner, Bradford and Hooker—
FRUIT PRODUCTION

Gardner, Bradford and Hooker—
ORCHARDING

Hayes and Garber—
BREEDING CROP PLANTS

Heald—
PLANT DISEASES

Horlacher—
SHEEP PRODUCTION

Hutcheson and Wolfe—
FIELD CROPS

Jones and Rosa—
TRUCK CROP PLANTS

Loeb—
REGENERATION

Löhnis and Fred—
AGRICULTURAL BACTERIOLOGY

Piper and Morse—
THE SOYBEAN

Rice—
THE BREEDING AND IMPROVEMENT OF FARM ANIMALS

Sharp—
CYTOLOGY

Sinnott—
BOTANY

Sinnott—
LABORATORY MANUAL FOR ELEMENTARY BOTANY

Sinnott and Dunn—
PRINCIPLES OF GENETICS

Thatcher—
CHEMISTRY OF PLANT LIFE

Thompson—
VEGETABLE CROPS

Weaver—
ROOT DEVELOPMENT OF FIELD CROPS

Weaver and Bruner—
ROOT DEVELOPMENT OF VEGETABLE CROPS

These two series were originally published as one under the title of McGraw-Hill Agricultural and Biological Publications. The late Dr. C. V. Piper was Consulting Editor from the inception of this series in 1917 until his death in 1926.

TEXTBOOK OF COMPARATIVE PHYSIOLOGY

BY

CHARLES GARDNER ROGERS, PH. D., SC. D.

Professor of Comparative Physiology in Oberlin College

FIRST EDITION
SECOND IMPRESSION

LANE LIBRARY

McGRAW-HILL BOOK COMPANY, INC.
NEW YORK: 370 SEVENTH AVENUE
LONDON: 6 & 8 BOUVERIE ST.; E. C. 4
1927

Copyright, 1927, by the
McGraw-Hill Book Company, Inc.

PRINTED IN THE UNITED STATES OF AMERICA

LANE LIBRARY

THE MAPLE PRESS COMPANY, YORK, PA.

TO

R. H. R.

PREFACE

More than a half century ago H. Milne Edwards, lecturing before the students of the University of Paris upon the Physiology and Comparative Anatomy of Man and Animals, furnished an ideal of physiological teaching which has to a certain extent been lost, and to which it seems desirable to return. His grasp of the subject was broad, well balanced, and far-reaching in its significance. The high degree of specialization of the biological sciences in more recent days, through attention to almost endless detail, particularly upon the morphological and medical sides, has narrowed the mental field of vision and distorted the normal perspective of the subject.

Physiology has not had the same attraction for the undergraduate student, nor the same favorable opportunity for growth that has been enjoyed by the other biological sciences. Failure to appreciate the physiological point of view, associated with the great emphasis placed by zoologists upon purely morphological works, the lack of trained teachers and of suitable texts, the lack of information as to sources of material, sometimes lack of funds to provide for practicable experimental work, and too close adherence to medical tradition have all contributed to this situation.

This volume is the outgrowth of a course in comparative physiology which has for a number of years been offered to students in Oberlin College. Their response to the general method of presentation has encouraged the author to put the material into a more generally accessible form. It is the purpose of this volume to help modify some of the existing conditions, to assist in the return to the broader view of physiology, and to offer to biological students with an elementary knowledge of physics and chemistry a cultural background of physiological ideas and ideals not to be gained from a study of technical mammalian physiology alone. The physiology of animals is really functional zoology. In so far as the author is aware, no other textbook of college grade approaches the subject from this standpoint. Since it is functional zoology it concerns itself with the primary functions of animals of all groups, especially of the invertebrates which constitute probably not far from 93 per cent of all known species of animals. No attempt is made to follow the stereotyped form and content of medical physiology. On the contrary, every attempt is made to give to the student definite ideas of the fundamental unity of inorganic and organic worlds, of a similar unity of life and of animal functions in

96281

different animal groups, of some conception of evolutionary changes in animal functions as well as in animal structures, of the fundamental significance of function as related to form among animals, and of some physiological bases of animal relationship.

Lack of space makes it impossible to include all the material that some may desire. Certain topics have been selected as worthy of emphasis, and others treated lightly or omitted. The topics selected have been found, during the past ten years and more, to stimulate thought and interest and to arouse the scientific imagination. Especially does it not seem practicable to include in this volume directions for laboratory studies. Such studies constitute the major portion of the course as it has been given by the author during the past years. If, at some later date, there shall seem to be a demand for laboratory material it may be supplied.

Failure to make due acknowledgment to any author or publisher is unintentional, but is explicable by the large number of works consulted. Recognition is due the many investigators whose work has been placed at the author's disposal. Permission to use copyrighted material has been granted by The Cambridge Press; Henry Holt and Company; Longmans, Green and Company of London; The Macmillan Company; G. P. Putnam's Sons; W. B. Saunders Company; The University of Chicago Press; The Yale University Press; and The McGraw-Hill Book Company, Inc. To all of these the author expresses his sincere thanks. Acknowledgment of indebtedness to such publications as Winterstein's Handbuch der Vergleichenden Physiologie, Von Fürth's Vergleichende Chemische Physiologie der Niederen Tiere, etc., is gladly made. Thanks are due to Prof. P. H. Mitchell, Prof. L. W. Sharp, and Dr. R. E. Snodgrass for permission to use published figures; to Prof. F. H. Pike and Prof. A. F. Shull for criticisms and suggestions; to Mrs. Helen Daniels Young for most of the pen and ink sketches from which the cuts were made; and to Mrs. Rose Humann Rogers for careful translations from foreign periodicals and for critical reading of the manuscript.

The author will be glad to receive corrections and criticisms from those interested.

C. G. R.

Oberlin, Ohio
December, 1926.

CONTENTS

PAGE

PREFACE vii

CHAPTER I

INTRODUCTION 1

The Physiological Point of View—Physiology Defined—Observational and Exact Sciences—Physiology Classified—Scope of Physiology—Relations of Physiology to Other Biological Sciences—Relations of Physiology to the Physical Sciences—Aims of Comparative Physiology—Structure of Matter—Electron and Atom—Molecules—Compound Substances—Energy and Energetics—Energy Transformers—Physical and Chemical Laws in Physiology; Continuity, Conservation of Energy, Conservation of Matter, Definite Proportions, Multiple Proportions, Mass Action, Laws of Thermodynamics, Gas Laws

CHAPTER II

SOLUTIONS 13

Solutions Defined—Water, the Principal Solvent, and an Electrolyte—Classification of Solutions—Crystalloidal Solutions—Colloidal Solutions—Electrolytic Solutions—Dissociation—Degree of Dissociation—Colloids—Physical States of Matter and Their Combinations—Heterogeneous Systems—Sols and Gels—Suspensions and Suspensoids—Brownian Movement—Physical Properties of Emulsions and Emulsoids—Adsorption—Combinations between Colloids and Other Substances—Sizes of Colloidal Particles—Osmotic Pressure of Colloids—Molecular Weights of Colloids—Molecular Weights of Certain Proteins—Colloids in Physiology; Carbohydrates, Fats and Lipoids, Proteins—Physical Properties of Proteins—Chemical Properties of Proteins—Color Tests—Classification of Proteins—Colloidal Behavior of Proteins—The Donnan Equilibrium

CHAPTER III

DIFFUSION AND OSMOSIS 39

Diffusion—Mutual Inter-diffusion—Membranes—Osmosis—What is Osmotic Pressure? Haldane's Explanation—Determination of Osmotic Pressure—Cryoscopy—Table of Osmotic Pressures of Solutions in Atmospheres—Osmotic Pressure of Tissues—Permeable and Semi-permeable Membranes—Factors Influencing Permeability—Direct Determination of Permeability—Applications of Dialysis and Osmosis in Physiology

CHAPTER IV

PROTOPLASM 52

General Appearance and Properties of Protoplasm—Structure of Protoplasm; Reticular and Alveolar Theories—Evidence as to the Structure of Protoplasm from Micro-dissection—Protoplasm a Hydrophyllic Emulsion

PAGE

Colloid—Reversals of Phase—Chemical Composition of Living Matter—Biogen Hypothesis—Protoplasm, an Heterogeneous System

CHAPTER V

PROPERTIES OF PROTOPLASM 63
Diagnostic Characters of Protoplasm; Chemical Composition, Metabolism, Cyclic Changes, Catalytic Agents—Fundamental Properties of Living Matter—Irritability; Classification of Stimuli, Nature of Irritability, Loeb's Theory of Irritability, Lillie's Theory of Irritability—Conductivity—Contractility—Spontaneity—Metabolism—Rhythmicity—Maintenance of High Temperature—Growth—Consciousness and Memory—Reproduction

CHAPTER VI

THE CELL 72
The Cell Theory—Elementary Cell Structure—The Nucleus—Functions of the Nucleus—The Cytoplasm—The Cell Membrane—Cellular Differentiation—Adaptation of Form to Function—Interactions of Cells—Special and General Functions of Cells—Cellular Modifications to Facilitate Movement—Cellular Modifications to Facilitate Secretion and Adsorption—Cellular Modifications to Facilitate Support and Protection—Cellular Modifications to Facilitate Storage and Insulation—Cellular Modifications to Facilitate Reception, Conduction and Storage of Impulses—Cellular Modifications to Facilitate Reproduction—Cell Division

CHAPTER VII

THE GENERAL PHENOMENA OF LIFE 85
Metabolism—Energy Transformations—Changes of Form—Conditions Essential for Production and Maintenance of Life—Factors Conditioning Life—Heat, Light, Water, Carbon Dioxide, Oxygen, Colloidal Condition, Hydrogen Ion, Special Conditions—Origin of Living Matter—Life, Definitions—Mechanistic and Vitalistic Conceptions of Life—Duty of the Scientific Observer

CHAPTER VIII

ORGAN SYSTEMS 94
Tissues and Organs—Morphological and Physiological Classification of Tissues—Organ Systems—Alimentary System—Circulatory or Transport System—Respiratory System—Circulating Fluid—Excretory System—Nervous System—Reproductive System

CHAPTER IX

THE TRANSPORT SYSTEM 106
The Internal Medium and Its Circulation—Need for a Circulating Medium Classification of Circulating Media—Functions of Blood—Quantity of Blood in the Body—Structure of Internal Media—Protozoa—Metazoa—Coelenterata—Echinodermata—Worms—Crustacea—Mollusca—Myriopoda—Insecta—Ascidia—Vertebrata—Dimensions, Numbers, and Areas of Red Blood Corpuscles—Dissociation of Oxyhaemoglobin—Evolution of the Red Corpuscles—Leucocytes—Thrombocytes

CHAPTER X

THE BLOOD AS AN OXYGEN CARRIER 124
Need of Special Oxygen Carriers Solubility of Oxygen in Water—Respira-

PAGE

tory or Chromoproteins—Chlorocruorin—Haemoglobin: Its Physicochemical Character—The Transport of Haemoglobin in the Body—Amount of Haemoglobin in the Blood—Haemoglobin Crystals—Haemin Crystals and Their Significance—Haemoglobin among Worms, Echinodermata, Mollusca and Crustacea—Significance of Haemoglobin in Lower Forms—Role of Haemoglobin as a Carbon Dioxide Carrier—Haemocyanin—Distribution of Haemocyanin—Properties of Haemocyanin—Crystallization of Haemocyanin—Spectral Properties of Haemocyanin—Analyses of Haemocyanin—Role of Haemocyanin as an Oxygen Carrier—Importance of Copper in Haemocyanin—Are There Many Haemocyanins?—Oxygen and Carbon Dioxide Carrying Powers of Different Bloods

CHAPTER XI

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE FLUID MEDIUM 142
Ancestry of the Fluid Medium—Freezing Point Determinations of Body Fluids—Ionic Phase of the Sea—Chemical Composition of Bloods of Invertebrates—Correspondences between Body Fluids and Sea Water; Molecular Concentration and Osmotic Pressure, Relative Concentration of Salts—Physiologically Balanced Solutions—McClendon's See Water—Van't Hoff's Solution—Ringer's, Locke's, and Other Solutions—Importance of Metallic Ions in the Blood—Correspondence in Temperature—Correspondence in Hydrogen Ion Concentration—Sørenson's Nomenclature—Hydrogen Ion Concentration of Body Fluids—Table of pH Values Expressed in H′ ion Normality—Buffer Substances of the Blood and Carbon Dioxide Transportation—Carbon Dioxide in the Blood of Marine Invertebrates

CHAPTER XII

COAGULATION OF THE BLOOD AND PHAGOCYTOSIS 165
Significance of Coagulation for Preventing Loss—Coagulation among Echinodermata, Mollusca, Crustacea, and in Limulus—Zoological Significance—Frog's Blood and Its Coagulation—Coagulation of Mammalian Blood—Theories of Coagulation—Phagocytosis—Fates of Foreign Particles in the Blood—Conditions Affecting Phagocytosis—Phenomena of Phagocytosis, Physical in Character—Fenn's Studies on Phagocytosis—Chance of Collision between Particles and Cells—Effects of Temperature—Surface Tension

CHAPTER XIII

CIRCULATORY MECHANISMS. 176
Need for Circulatory Mechanisms—Mechanism for Secretion of Internal Fluid—Circulation among Protozoa—Circulation among Sponges—Factors Involved in the Evolution of Vascular Systems—Development of Vascular Apparatus Due to Physiological Demand—Types of Vascular Apparatus—Components of Vascular System of an Individual—Historical Statement as to Circulation of Blood—Incomplete Systems—Functions of Different Parts of Vascular System; Heart, Primary Function of Heart, Normal Contraction of Frog's Heart, Capillaries, Rouget Cells, Sinuses, and Veins—The Elastic Tube System—Peripheral Resistance

CHAPTER XIV

PHYSIOLOGY OF THE HEART. 192
Relation of Oxygen to Heart Beat—Effects of Changes of Temperature

PAGE

upon Heart Action—Temperature Coefficient—Origin of Heart Beat—Myogenic Theory of Heart Action—Evidence from Tissue Cultures and from Embryology—The Importance of Inorganic Salts—Importance of Products of Heart Tissue Metabolism—Importance of Electrical Charges of Membranes—Importance of Hydrogen Ion Concentration—Heart Block—Neurogenic Theory of Heart Beat—The Pace Maker of the Heart

CHAPTER XV

PHYSIOLOGY OF THE HEART (Continued) 208
Cardio-regulator Nerves of Invertebrates; Mollusca, Arthropoda—Cardio-regulator Nerves of Vertebrates—Heat Production by Heart Muscle—Starling's Law of the Heart

CHAPTER XVI

RESPIRATION 218
Meaning of the Term—External and Internal Respiration—The Need for Oxygen—Historical Statement—Organs of Respiration—Types of Respiratory Structures—Gas Laws—Tension of a Gas—Absorption of a Gas by a Liquid—Water as a Respiratory Medium—Distribution Curves of Oxygen, Carbon Dioxide, and Temperature in Finger Lakes of Central New York—Variations in the Oxygen Content of Water under Special Conditions—Air in the Depths of the Ocean—Aerobic and Anaerobic Respiration—Oxygen Tension in Tissue

CHAPTER XVII

RESPIRATION (Continued) 234
Respiration in Protozoa—Comparison of Respiration in Various Groups of Animals—Oxygen Consumption and Oxygen Concentration—Respiration a Function of the Rate of Diffusion—Respiration in Worms—Gas Exchange of Anodonta—Respiratory Processes of Mya and Other Marine Mollusca—The Crystalline Style as a Factor in Respiration—Respiration among Crustaceans—Respiratory Surface in Crabs—Respiration in Insecta—Respiratory Exchanges of Lower Land Animals—Respiration of Diving Beetles—Respiration of the Grasshopper—Respiration among the Bees—Oxygen Tension in the Tissues of Certain Invertebrates—Respiratory Exchange of Aquatic Animals—Respiration of Fishes—Oxygen Requirements of Fish—Effects of Carbon Dioxide—Dissociation of Blood of Fishes—Functions of the Swim Bladder in Fishes—Respiration of Hibernating Animals—Rate of Oxidation in Echinoderm Eggs—Oxygen Consumption during Development—Respiratory Exchange of the Lower Animals at Different Temperatures—The Flow of Oxygen through the Respiratory Epithelium—Relation of Hydrogen Ion to Respiration

CHAPTER XVIII

CATALYTIC ACTIONS OF ANIMALS 261
Bayliss' Mechanical Schema of Catalysis—Characteristics of Catalysts—Mode of Action of Catalysts—Balanced Actions—Enzymes as Catalysts—Yeast Fermentation—Endo- and Ecto-enzymes—Hydrolysis—Characteristics of Enzymes Similar to Those of Inorganic Catalysts—Chemical Nature of Enzymes—General Properties of Enzymes—Zymogens or Proferments—Activators and Pro-enzymes—Co-enzymes—Zymogens in Protozoa

PAGE

CHAPTER XIX

THE WORK OF DIGESTION 270
The Purpose of Digestion—Intracellular Digestion—Character of Food Supply Demanded—Peptic Digestion—Digestion by Trypsin and Erepsin—Effect of Temperature upon Protein Digestion—Hydrogen Ion Favorable for Digestion—Specificity of Enzymes—Protein Synthesis and Specificity—The Amino Acids—Fat Digestion, Reversible Action of Lipase—Physiological Significance of Reversibility of Lipase Action—Synthetic Enzymes—Actions of Enzymes upon Carbohydrates—Starch Digestion—Classification of the More Common Enzymes—Distribution of Enzymes in the Animal Kingdom—Enzymes of Invertebrates—Roaf's Method for Studying Digestive Activity—Universal Occurrence of Enzymes among Animals—Evolution of Digestive Enzymes—Rennase

CHAPTER XX

SECRETION 287
Meaning of Term—Glands—Typical Gland Structures—Types of Work of Glands—Secretion a Sign of Irritability—Phases of Secretory Process—How is Secretion Induced?—Secretion Through Nerve Stimulation—Role of Muscle Fibers in Gland Action—Secretion Through Chemical Stimulation—Kinds of Substances Secreted—Period of Discharge—Period of Recovery—Changes of Gland Cells during Secretion—Change of Permeability during Discharge

CHAPTER XXI

THE NUTRITION OF ANIMALS 296
Purpose of Nutrition—Differences between Living and Lifeless Machines—Elements Demanded in a Food Supply—Complexity of Food Stuffs Needed—The Nitrogen-free Diet of Termites—Chemical Cycles in Nature—The Water Cycle—The Carbon Cycle—The Oxygen Cycle—The Nitrogen Cycle—The Phosphorous Cycle—Foods—Brief Classification of Food Substances—Water as Food—Inorganic Salts as Food—The Vitamins

CHAPTER XXII

NUTRITION OF DIFFERENT ANIMAL GROUPS 308
Nutrition of the Protozoa—Food of Rhizopods—Feeding Experiments upon Protozoa—Peptic Digestion of Myxomycetes—Food Vacuoles, Reactions—Starch Digestion—Amoeba, Historical Accounts of Feeding—Tryptic Digestion in Amoeba—Digestion of Starches and Fats—Food Taking and Nutrition of Infusoria—Nutrition of Sponges—Nourishment of Aquatic Animals by Matters Dissolved in the Water—Food Taking by Sponges—Intracellular Digestion—Enzymes of Sponges—Nutrition among Coelenterates—Tryptic Digestion of Coelenterates—Intra-cellular Digestion among Coelenterates—Contact Digestion—Gland Cells and Their Functions—Digestion among the Alcyonaria—Digestion among Worms—Comparison of Digestion of Worms and Coelenterates—Digestion in Planaria—Effects of Parasitism upon Digestion—Functions of Mid-gut of Worms—Digestion among Crustacea—The Crystalline Style—Rate of Digestion in Cold Blooded Animals

CHAPTER XXIII

ABSORPTION 334
Fat Absorption Possible Methods of Fat Absorption Historical Statement

PAGE

—Experiments upon Fat Absorption—Absorption of Carbo-hydrates and Proteins

CHAPTER XXIV

UTILIZATION OF FOOD MATERIALS IN THE BODY 342
Metabolism—Factors Determining the Value of Protein Food—Adequate and Inadequate Protein Food—Percentage Composition of Proteins—Biologic Values of Proteins—Nitrogen Equilibrium—Carbon Equilibrium—Complete Balance Experiments—Carbohydrate Metabolism of Animals—Glycogen Formation and Use—Blood-sugar—Occurrence of Glycogen in Lower Animals—Functions of Sugar in the Animal Body—Utilization of Fats in the Body—Animal Heat—Poikilothermous Animals—Homoiothermous Animals—Variation of Temperature of Man and Other Animals—Why is the Homoiothermous Condition Desirable?—Heat Production and Heat Loss—Factors Influencing Heat Production—Specific Dynamic Action of Protein Food—Heat Centers and Heat Nerves—Body Mass and Body Surface Factors in Temperature Regulation—Relation of Cell Surface to Body Surface—Factors Regulating Heat Loss -Physiological Oxidation

CHAPTER XXV

ENDOCRINE GLANDS AND THEIR WORK. 361
Glands of Internal Secretion—Nature of Endocrine Secretions—The Role of the Endocrines—The Thymus Gland—The Thyroid Gland—The Adrenal Gland—Adrenalin in Annelids—The Pituitary Gland—The Parathyroid Glands—The Pancreas—The Pineal Gland

CHAPTER XXVI

PHYSIOLOGY OF MOVEMENT. 373
Streaming Movements—Amoeboid Movement—Cilia—Structural Basis of Coordinated Ciliary Action—Variations in the Irritability of Ciliated Cells—Effects of Ions on Ciliary Movement—Effect of Hydrogen Ion Concentration—Effect of Temperature upon Ciliary Action—Effect of Osmotic Pressure—Comparison of Ciliary and Muscular Action—Reversal of Ciliary Action—Undulating Membranes—The Graphic Method—Fundamental Physiological Properties of Muscle—Muscular Movements—Distribution of Contractile Elements in the Animal Kingdom—Structure of Striated Muscle—Extensibility and Elasticity of Muscle—Muscle Action—Forms of Muscle Contraction—The Muscle Twitch—The Latent Period—Effect of Temperature upon Muscular Contraction—Tonicity of Muscle—Staircase Contraction—Summation of Contractions and the Development of Tetanus—The All-or-None Law—Muscular Fatigue—The Osmotic Pressure of Resting and Fatigued Muscle—Determination of the Presence of Lactic Acid in Muscle—Heat Production in Muscle—Summary of Changes Taking Place during the Muscle Twitch—Red and White Muscle

CHAPTER XXVII

EXCRETION. 412
Excretion Defined—Excretion among Protozoa—Excretion among Metazoa—Excretion among Sponges and Coelenterates—Superficial Intracellular Excretion—Storage Excretion—Localized Excretion—Glandular Excretion—Excretion among Echinodermata—Excretion by Diffusion—Intracellular Excretion—Storage Excretion—Excretion among Worms—Correla-

PAGE

tion between Types of Excretory Systems and Circulatory Systems and Fluids—Arrangements of Excretory Organs among Worms—Types of Filtering Mechanisms—Types of Arrangement of Excretory Surfaces—Rate of Excretion—Functions of Different Parts of Nephridia—Excretophores—Fate of Excretophore Substances—Excretion among Mollusca—Excretion among Crustacea—Mode of Excretion—Excretion by Fat Bodies—Excretion among Insects—Theories of Kidney Action—Absorption by Kidney Tubule—Threshold Bodies

CHAPTER XXVIII

THE NERVOUS SYSTEM. 446

Differentiation Leading to Nervous Structures—Need for a Nervous System—Behavior—Classification of Forces Acting Upon an Organism—Vitalistic View of Animal Behavior—Mechanistic View of Animal Behavior—Neuromuscular Apparatus of Protozoa—Nervous Organization—The Nerve-net—Transition from Nerve-net to Synaptic System—Chemical Differentiation in the Nervous Systems of Invertebrates—The Neurone Theory—The Synapse—Integration of Animal Bodies—Range of Ethereal Vibrations—Classes of Receptors and Their Limitations—Threshold of Stimulation—Law of Specific Nerve Energies—Elementary Functions of Excitable Tissue—Carbon Dioxide Production by Nerve—Fatigue and Exhaustion—Nature of Nerve Action—Rate of Discharge of Motor Nerve Cells—The Nerve Impulse—Lucas' Gas Chamber Experiment—The All-or-Nothing Law—The Refractory Period—Temperature Coefficient of Refractory Period—The Local Excitatory Process—The Propagated Disturbance—Rate of Conduction of Propagated Disturbance—The Reflex Mechanism—Conduction in Peripheral Nerve and in Reflex Arc Compared; Rate, After-discharge, Summation, Irreversibility, Fatigue, Variability of Threshold, Reinforcement, Inhibition—Reciprocal Inhibition of Antagonistic Muscles—Types of Nervous Organization—Primitive Receptor-effector Mechanisms of Sponges—Types of Migration of Cell Body on Neurone—Nervous Mechanisms of Coelenterata—Rhythmical Pulsations of Medusae—Rate of Transmission in Metridium—Nervous Systems of Echinodermata and Their Actions—Righting Movements of Starfish—Reciprocal Inhibition in Antedon—Nervous Systems of Worms—Significance of Polarity in Nervous Organization—Specialization of Nerves among Worms—Receptor Organs of Earthworm—Physiological Dominance in Worms—Nerve-net and Nerve-plexus—Nervous System of Earthworm—Sub-epidermal Nerve Plexus—The Central Nervous System—Peripheral Nerves—Minute Structure of Ventral Nerve Cord—Minute Structure of Ventral Ganglion—Functions of Neuropile—Operation of Nervous System of Earthworm—Comparison of Reactions of Earthworm and Marine Worm—Bovard's Studies upon Earthworm—Reciprocal Innervation in Earthworm—Summary of Rates of Transmission in Worms—Muscle Tension and Reflexes in Earthworm and Nereis—The Homostrophic Reflex in Earthworm—The Homostrophic Reflex in Diplopods—Nervous Organization among Arthropoda—Nervous System of Branchipus—Nervous System of Corydalis cornuta—Nervous System of the Crayfish—Experiments upon Sicyonia—The Nervous System of Limulus—Experiments upon Limulus—Control of Respiratory Mechanism—Nervous System of the Honey Bee

PAGE

CHAPTER XXIX

PHYSIOLOGY OF REPRODUCTION 534

Significance of Reproduction—Biogenesis—Fundamental Types of Reproduction—Asexual Reproduction—Sexual Reproduction—Reproduction among Metazoa a Series of Phenomena—The Egg Cell a Highly Specialized Cell—The Germ Cells—Forms of Mature Spermatozoa—Egg and Sperm Shedding—Factors Involved in Development—Fertilization—Effect of Hydrogen Ion upon Fertilization—Theories of Fertilization—Number of Spermatozoa Required for Fertilization—Heat Production during Fertilization and Early Cleavage—Artificial Parthenogenesis—Parthenogenetic Development in Nature—Physiological Evasions of the Hazards of Development—Types of Cleavage—Development a Series of Interactions—Effects of External Factors upon Development; Pressure, Osmotic Pressure, Temperature, Isolation of Blastomeres, Variations in Chemical Environment, Effects of Oxygen, Effects of Carbon Dioxide, Effects of Sodium and Calcium, Effects of Potassium, Effects of the Sulphion, Effects of Combinations of Salts—Effects of Internal Factors upon Development; Formative Substances or Hormones, Chromosomes

BIBLIOGRAPHY 579

INDEX 609

TEXTBOOK OF COMPARATIVE PHYSIOLOGY

CHAPTER I

INTRODUCTION

In beginning the study of any new subject it is usually desirable for the sake of economy of time to have some rather definite ideas as to the bounds and limits of the fields to be examined and also definitions of the terms involved. In such a book as this it is impossible to do more than gain a somewhat limited view of a small part of the ground that might be covered. In addition to the fact that the subject is naturally a very comprehensive one, there is the more important consideration that it is constantly growing. The amount of new knowledge which is every year being added to the store of information makes it almost impossible to keep pace with its natural growth.

Physiological Point of View.—It is frequently difficult for one who is just beginning the study of physiology to get what may be called the *physiological point of view*. The reason is fairly evident, though not less to be deplored because easily recognized. Most of the biological courses given to undergraduates in our colleges stress the form and structure of animals rather than the functions. Zoölogy, in many a laboratory, has meant merely the study of the corpses of animals. In place of the living, dynamic forms, students have spent their laboratory hours in the consideration of embalmed specimens. This has gone on to such an extent that many a student has failed to realize that these lifeless forms were at one time living, acting, dynamic things. It is now necessary to think in terms of function—of the organism as doing something—and to think of form and structure only as contributing to an understanding of the function under consideration. The work which the organ does is more fundamental than the organ which does the work. There are many instances in the animal kingdom in which a common function is being performed in different types of animals by organs of very different structure, but which have gone through a long evolutionary development to bring them to their present degree of efficiency.

The term *physiology*, derived from two Greek words φύσις and λόγος, meaning nature and speech respectively, is defined as "the sum of knowl-

edge concerning the functions of living things." This definition involves more than is frequently conceded even by those who are in a position to appreciate the breadth of the subject. This rapidly increasing sum of knowledge makes it necessary from time to time to revise many of the surmises concerning the nature of physiological processes. The added information and the rearrangement of information already at hand involve not only the study of living things themselves and a survey of the ways in which their different parts operate, but call for an understanding of physical and chemical laws, forces, and operations which only a few years ago were considered to have little or no bearing upon the subject—if they were even known at all. In fact, it is hardly too much to say that a complete understanding of the functions of a living organism would involve not only the content of physiology but a knowledge of the elements of physics and chemistry as well.

Observational and Exact Sciences.—Every science, in the course of its growth, passes through certain states before it comes to be recognized as an exact science. In Astronomy, Physics, and Chemistry there was at first simply the casual observation of the more apparent facts, followed by a growing appreciation of the truth that the facts observed formed parts of a perfectly ordered system of truth in accord with definite laws. Then came the more careful and accurate observations upon which statements of the controlling laws could be formulated. Such statements required the most exacting examination and criticism—a work which, even in the sciences mentioned, is not yet complete. So in biology, and especially in physiology, there has been a somewhat delayed advance along similar lines. Biology has been spoken of as an *observational science* because most of the work done in it has been of the descriptive sort, but here, too, there has been growing recognition of the fact that living organisms, in their varied activities, are just as truly subject to laws as is matter in its non-living forms. Physiologists are now engaged in the process of reducing, so far as possible, physiological processes to physical, chemical, and mathematical expressions. There seems no good reason to suppose that physiology may not take its place as an *exact science*, for the phenomena studied and the reactions carried on appear more delicately balanced and controlled than any phenomena yet studied. The most serious obstacles to progress in physiology appear to be prejudice and superstition. With these cast aside, great advances in our understanding of the nature and operations of the living substance should easily be made.

Physiology Classified.—The subject of physiology itself may be subdivided and classified. References may be made to *special physiology*, *i.e.*, the functioning of some particular organ or tissue; to *general physiology*, that branch of the subject which deals with the fundamental operations of all types of living matter in common; or to *comparative physiology*,

by which the comparative study of the functions of a variety of organisms is meant. It will be readily seen that the ground covered by the last two terms is very much the same.

The Scope of Physiology.—It has been and is still, in many quarters, held that the study of physiology is and should be limited to the highest organisms—mammals, including man. This seems a questionable notion, and while at this place no attempt will be made to argue concerning the relative merits of the higher and the lower organisms as objects of physiological investigation, it will be held that the subject of this book has to do with the workings of the vital machine, whether it be simple or complex, Amœba or man. The subject includes all those activities concerned with the maintenance of the protoplasmic structure, the taking in of food, the building up of protoplasm, the carrying on of a wide range of chemical reactions at low temperatures in the processes of metabolism, the energy transformations taking place within the organisms, the elimination of wastes, and the segregation and separation of protoplasmic units capable of independent existence. It includes all the activities of the organism under definite or changing environmental conditions –its reactions to its environment. It includes also all the activities involved in the perpetuation and development of the race to which it belongs. The whole series of phenomena of heredity, variation, evolution, etc., which may be included under the term *racial physiology*, are physiological in nature and can never be solved until they are approached from this standpoint.

Relations of Physiology to Other Biological Sciences.—Biology, the science of life and of living things, is for convenience frequently divided into botany and zoölogy, each dealing with a grand division of living things. Each of these, in turn, may be subdivided into a series of sciences, anatomy, histology, cytology, embryology, physiology, etc., dealing respectively with grosser structure, minuter structure, cell structure and phenomena, individual development, and function.

It must be pointed out that physiology alone of the branches or subdivisions of the subject is especially concerned with the activities and functions of the living organism. Biology has, for the most part, concerned itself with the form and structure of the dead organism, and its tissues, and has had until recently comparatively little to do with the deeper problems of life. The study of what the organism does and the way in which it accomplishes its task is reserved for the student of the living organism. To him biology becomes alive, directly in proportion as he applies his endeavors to making clear the various processes employed by the animal or plant in its daily life.

The present-day student of physiology has come to realize that living matter is a substance subject to the same laws and forces as all other parts of nature. Some of these processes of the animal or plant body are

easily demonstrated to be chemical in nature. Others, upon closer study, are also found to be chemical. Still others are found to be purely physical processes; while still others, although subjected to much investigation, have not yet revealed their inner nature. Continued and careful study is constantly adding to the list of the phenomena of the living body which are definitely physical or chemical. The machine responsible for carrying on these reactions is made of material stuffs, aggregated, to be sure, in a complex and peculiar way, but so far as it is possible to see subject to all the physical and chemical laws that hold in the case of other forms of matter. Up to the present time it has been impossible to identify or determine the existence of any force acting within the living organism which does not operate in accordance with physical and chemical laws. So it may be said that the dynamic processes of the organism are, so far can at present be determined, chemical and physical in character, and that the materials composing the machine are not essentially different from those which compose other known substances.

The Relation of Physiology to the Physical Sciences.—Living matter is made up of molecules, molecules of atoms, and atoms of electrons. These ultimate units are the same, so far as is known, for all sorts of matter. In living matter these ultimate units are seen arranged into their most complex aggregates. These formulations are constantly being modified through additions, subtractions, and rearrangements. The materials involved are the materials of chemistry and physics. The forces employed or set free in all of these manifestations are the forces of chemistry and physics. The processes employed in effecting these changes in substance appear not to be different in kind from those of chemistry and physics. So it appears that the problems of physiology, dealing as they do with physical and chemical phenomena, must be attacked by the methods of the physicist and the chemist and solved and explained in terms understandable by them. Ultimate explanations in the realm of physiology will be difficult to obtain, simply because of the limitations of physics and chemistry.

The Aims of Comparative Physiology.—The aims of comparative physiology are:

1. To give an exact and adequate conception of the essential nature of the various processes of the organism;

2. To make a comparison of these processes in different types of animals to discover whether they really operate under the same fundamental laws;

3. To trace, if possible, the course of evolution of these processes from the more generalized to the more specialized and complex;

4. To gain some idea, from the similarity of method employed by different groups of animals, of the possible relationships existing between them; and

5. To show the correlation and integration of the various parts and processes in the economy of the individual.

Dr. Loeb has summed up the aim of physiology in these words: "To visualize all phenomena of life in terms of groupings and displacements of ultimate particles." Verworn has stated the same idea in different words: "To find out what the vital process is, to determine the exact cause of vital phenomena."

It will be evident that the clearest understanding of any vital process may be gained from the study of those forms in which it appears on the whole best developed and where it is least attended by coincident obscuring phenomena. The protozoa are, of course, the simplest animals so far as complexity of structure is concerned, but here each vital process is accompanied and partially obscured by other vital phenomena, the single mass of protoplasm being engaged in doing a number of things at the same time. Such forms, therefore, instead of being most simple are from the physiological point of view most complex. For our chief studies therefore, forms must be chosen in which each individual process can be so clearly isolated as to give unconfused information as to what is really taking place. The forms in which the processes show themselves to the best advantage are, in general, those in which specialization has gone on to a considerable degree. When, from a study of these more specialized types, the laws and underlying principles of the various physiological processes have been discovered, it is possible to test out the workings of these laws and principles in the less specialized types of structure.

It may be argued that one is not in a position to understand the workings of the machine until he is familiar with all its parts and their relations to each other. The work of any highly complex machine is an enigma to the uninitiated. One may even make a careful study of the parts of the machine and their relations to each other without acquiring any adequate conception of the working of the machine. It is when the machine is in operation that one has the best opportunity to discover its real intent. It may not be possible to determine, while the machine is in operation, some of the minutiæ as to the structure of its given parts, but it will be possible to get some idea of the work which the machine is doing, and of the manner in which all parts of the machine contribute to the total result. Similarly, it is much easier for one to get a proper conception of the organism and of the work which it is doing from a study of that organism while it is doing its work than from a study of the minute details of structure which the organism presents.

The Structure of Matter.—For more than 2500 years men interested in metaphysics have speculated concerning the ultimate constitution of matter. Ancient Hindu and Greek philosophers held that the physical universe (or, as sometimes taught, the whole universe, both physical and mental) is composed of very small indivisible particles or atoms which

are in constant motion. The first clear statements of this idea came in the writings of Leucippus and Democritus, who taught that all phenomena are to be explained by the incessant movements of atoms, which differ only in shape, order, and position.

The beginnings of a scientific understanding of the structure of matter date back only to the work of Lomonossoff, a Russian physical chemist (1743), and to that of John Dalton, an Englishman (1803–1807), who has given to us the basis of the modern atomic theory. The Russian work was to all practical purposes buried and unknown, and was resurrected only in 1904. Dalton's work has played a most important part in correlating and interpreting the known facts of chemistry. Dalton states that all material substances are composed of minute particles or atoms of a comparatively small number of kinds. All the atoms of the same kind have the same size, weight, and other properties.

The theory as at first stated has been developed, added to, and modified by the work of many investigators. During recent years there has been published a very great amount of scientific evidence of such a character as to establish beyond any reasonable doubt the facts of the atomic and molecular structure of matter. It is impossible here to trace the steps in the development of this bit of scientific truth, but merely to make a very simple and brief statement of the facts as they are known at the present time.

The Electron and the Atom.—Probably the most important addition to our information as to the structure of matter is the idea that the atom is not a simple indivisible thing. It has been shown to be a very complex system, whose components, subatoms or electrons, are in very rapid orbital motion. The electrons are of two kinds, positive and negative. These are alike in the strength of the electrical charge which they bear, but wholly different in mass. The negative electron is associated with a mass which is only $\frac{1}{1845}$ that of the hydrogen, the lightest known, atom. The positive electron appears never to be associated with a mass less than that of the hydrogen atom. The diameters of some of these atoms have been calculated and found to be, for helium, 2×10^{-8} centimeter; for the hydrogen atom, slightly less; and for oxygen and nitrogen atoms, slightly more. From investigations which have been made it appears that the atom is constructed somewhat upon the plan of a solar system, having at its center a nucleus bearing positive electrical charges and negative electrons whirling in orbits about it. The rate of movement of these negative electrons appears to approach closely that of light. The electronic constituents are as small in comparison with the dimensions of the atomic systems as are the sun and planets in comparison with the dimensions of the solar system. Of course, in such a system the electronic or other particles can occupy but a very small portion of the space enclosed within the system. Practically all the space is empty.

The numbers of electrons entering into the structure of an atom is a matter of interest and seems to be the distinguishing character between atoms of different sorts. For example, the hydrogen atom appears to be like a sun with a single planet—a positive and a negative electron, the two being equal electrically but differing in inertia, the positive being the more massive, though the more concentrated, of the two. Helium appears to have two central unbalanced positive charges and two revolving negative charges. The lithium atom has three of each; beryllium, four of each; boron, five of each; carbon, six of each; nitrogen, seven of each; oxygen, eight of each; and so on up to uranium, which has ninety-two positive electrons in its nucleus and as many negative electrons whirling in orbits about it. It also appears that in the larger atoms there are other positive and negative electrons in the nucleus serving to bind the positive electrons together, and possibly it may be the presence of these negative particles which accounts for the fact that the atomic weights of some of the atoms are higher than would seem to be necessary if there were not such binding electrons in the nucleus.

Molecules.—The atoms have the power to unite with each other under certain conditions and form larger bodies, called molecules. If the atoms entering into such a combination are all of the same sort, the product is a pure substance; if they are not all of the same kind, it is a mixture.

In an elementary substance, in which the atoms are all of the same kind, it is frequently found that the molecules are not all alike, being made up of one, two, three, four, or more atoms each, giving rise to what are known as *allotropic substances*. Or it may be that even if the substances are built up of molecules containing the same numbers of like atoms they will appear to be different because the atoms are differently arranged in the molecule. Or it may even happen that more than one kind of atom may enter into the structure of a molecule and still allow the display of nearly all the chemical and physical properties characteristic of a given substance. Such a condition would more naturally occur in the molecules of elements of high atomic weight, such as lead, or in the molecules of complex substances of organic origin.

It appears true also that some of the substances which have been, in the past, thought of as elements are really mixtures of substances, very nearly but not entirely identical. Such substances, *isotopes*, have been shown to exist. Table 1 indicates some of the isotopes known to chemists. This table does not pretend to be complete and is introduced simply to emphasize the fact that one need not be surprised to find at any point in the series of elements substances which are not exactly uniform in composition. If this is true among the substances which are thought of as elemental, how much more likely is it to be true of compounds, especially of the more complex substances, which are called organic.

TABLE 1.—ISOTOPES

Element	Number of isotopes	Atomic weight	Masses of isotopes
Lithium	2	6.94	7, 6
Boron	2	10.9	11, 10
Neon	2	20.2	20, 22
Magnesium	3	24.32	24, 25, 26
Silicon	3	28.1	28, 29 (30)
Chlorine	2	35.46	35, 37
Argon	2	39.9	40, 36
Potassium	2	39.10	39, 41
Calcium	2	40.07	40 (44)
Nickel	4	65.37	64, 66, 68, 70
Selenium	6	79.2	80, 78, 76, 82, 77, 74
Tin	8	118.7	120, 118, 116, 124, 119, 117, 122 (121)

Compound Substances.—As the name indicates, these are composed of atoms of different kinds entering into combination with each other. There is here the greatest possibility of combination between atoms of different sorts, and the fact that such combinations are formed is evidenced by the almost staggering number of compounds known. There is here also the possibility of the production of substances which appear different from each other even though they contain exactly the same atoms as are found in some other substance. The difference occurs in the manner in which the atoms are arranged in the molecule. Such substances, whose molecules are identical in composition but different in structure, are called *isomers*.

It has been customary to divide chemical compounds into inorganic and organic, basing the division upon the distinction between those compounds formed without the action of living cells and those formed by the action of living cells. This distinction has been replaced by the distinction which separates those which are compounds of carbon from those which are not carbon compounds. The fact that many carbon compounds have been elaborated in the laboratory appears to make tenable no longer the claim that carbon compounds are alone the products of living cells.

It appears very possible that when all the facts upon which to base a conclusion are at hand it will be found that there is no real distinction to be made between the organic and the inorganic—that there has been no discontinuity in the evolution of matter from the very simple to the very complex. Leibnitz stated many years ago the principle that there is no break in nature, and that nothing passes from one state to another without passing through all the intermediate states. The combinations of

electrons to form atoms, the combinations of atoms to form molecules of elementary and compound substances, and the various combinations of even complex molecules, all together probably form a continuous and harmonious evolutionary system.

Conditions upon the earth are now, doubtless, vastly different from what they were when carbon compounds first appeared in abundance. While the conditions which then existed, favoring the formation of such compounds, at first simple, then somewhat more complex, and then of continually increasing diversity, may not now exist, one must not state dogmatically that such compounds may not continue to be formed. It will, no doubt, be considered by many absurd to claim that the gap between the inorganic and the organic is to be bridged by natural processes under present conditions. The recent work of Millikan, the full significance of which is not yet apparent, seems to indicate the possibility that free electrons in space about the earth may even now be uniting to form atoms. Likewise the work of Benjamin Moore indicates that it is still too soon to take the position that substances which have long been considered as of organic origin may not be produced out of inorganic materials in a state of nature as the result of the catalytic activity of substances in the colloidal condition acting upon carbon dioxide and water in the presence of light. Creation thus comes to be a continuous process—even on the earth—and not an event which took place once and for all time at some particular remote period.

Energy and Energetics.—The two great realities with which the student of science has to deal are matter and energy. The term commonly used to distinguish force is *energy*, and by energy is meant the ability or the power to do work. It will be noted that the word energy involves the concept of motion, either existent or potential. Some of the earlier writers believed not only in the existence of forms of matter which had no common factor but in different manifestations of force, which were not and could not be related to each other. The necessary conclusion now is that not only is all matter composed of the same sorts of ultimate units, but also that all forms of energy have a common origin, the energy of the electron.

Energy is found to exist in two chief types:

1. Kinetic energy, the energy of motion.
2. Potential energy, the energy of position.

Energy of motion appears in a variety of forms, as electrical, magnetic, atomic, molecular, radiant, chemical, gravitational, mechanical, and thermal. It is possible that all these different manifestations may be explained upon the assumption of the energy of the electrons. It is certainly not difficult to transform one form of energy into another. Some authors would add to the list given *biotic energy*, as a distinct form of energy found only in living matter. This, too, may be derived from

the energy of the electrons which make up the living matter. At any rate, there is not at the present time sufficient reason for distinguishing this form of energy from the chemical energies of the substances which are to be found undergoing change in the living substance.

Energy of position may be that of a weight in an elevated position, which may, if properly harnessed, accomplish work when allowed to move; or it may be that of electricity accumulated in a storage battery, or the energy of chemical substances which may be released in the formation of some new chemical substances; or that of food substances which may be transformed, with the production of heat or light or electricity or the accomplishment of work; or that of storage substance in living cells, which, when drawn into the vortex of the metabolic activities of the cell, may furnish the energy for the performance of a great variety of cellular activities.

Energy of one form may, under suitable conditions, be converted into energy of another form. Thus, kinetic energy of moving air or of falling water may be converted into electrical energy. By the use of the proper type of transformer the electrical energy may be converted into mechanical, or thermal, or radiant energy. The energy of the sunlight may, in a similar way, be converted into mechanical energy, or it may be stored up by the green plant and form a supply of chemical potential energy. The energy of chemical compounds, bound in the molecules through the attractions of the different atoms for each other, may be transformed into heat energy or mechanical energy. In all these transformations of energy there appears to be a tendency for energy to be degraded into heat. All forms of energy may ultimately appear as heat, but it does not seem possible at present to convert heat energy into all the other forms of energy.

Energy Transformers.—Any physical system through which energy is transformed from one sort to another may be spoken of as a transformer. Such transformers are of great variety—some very simple, and some very complex. Many machines have been constructed as the result of human ingenuity. These serve in a mechanical way as energy transformers. Many energy transformers are the result of long-continued evolutionary processes, dealing in particular with what is commonly known as living matter. It has been customary to think of living cells as the most wonderful of these energy transformers, and to attribute to them very special powers and properties because of the things they have been observed to do. Living cells are themselves highly complex colloidal systems. As such they exhibit properties which are common to other colloidal systems. Among these properties is the ability to synthesize more complex substances out of their less complex components. The more there is discovered concerning the nature of the living substance the more likely it seems that the inorganic colloids are the substances

that must be looked to for the first appearance of those remarkable energy transformations commonly attributed to living cells. The very intimate way in which the components of the colloidal systems are related to each other and the great expanse of internal surface exposed render the colloidal condition ideal for transformations of both material and energy. Certain it is that some of the inorganic colloids, such as ferric hydrate, have the power to synthesize carbon dioxide and water into more complex molecules. It has been shown that the synthetic power of the chloroplast of the green plant is not due to the chlorophyll held in the chloroplast but to an iron compound of a simpler sort. The earliest forms of matter which had the power to synthesize even very complex substances, must have been very simple as compared with the simplest forms of living matter as known at the present time. The fact is also becoming apparent that in living cells chemical operations are initiated and controlled not by the active protoplasm as a whole, but by certain special substances, produced by the activity of the protoplasm, termed *enzymes*. These are the energy transformers of living matter.

Physical and Chemical Laws in Physiology.—The student of physiology must keep constantly in mind the fact that not only do the same elemental substances enter into the composition of living matter as compose non-living matter, but the materials of the living substance behave in accord with the same principles as are in force in the inorganic realm. Scientists have attempted to express these principles in the form of certain statements, called laws. Some of these laws are mentioned here:

1. *Continuity.*—The principle, enunciated by Leibnitz, that there is no break in nature, and that nothing passes from one state to another without passing through all the intermediate states.

2. *Conservation of Energy.*—The law that the total energy of any material system is a quantity which can be neither increased nor decreased by any action between the parts, though it may be transformed into any of the forms of energy

3. *Conservation of Matter.*—The principle that the total mass of any material system is neither increased nor diminished by any reaction between the parts.

4. *Definite Proportions.*—The principle that every definite compound always contains the same elements in the same proportions by weight, and if two or more elements form more than one compound with each other the relative proportions of each are fixed.

5. *Multiple Proportions.*—The law that when the same elements unite in more than one proportion, forming two or more different compounds, the higher proportions of the elements in the compounds are simple multiples of the lowest proportions, or the proportions are connected by some simple common factors.

6. *Mass Action.*—The law, established by Guldberg and Waage, that the chemical action of a reacting substance is proportional at any moment to its active mass. The active mass is usually considered as measured by the molecular concentration, but sometimes, for dissolved substances, by the osmotic pressure.

7. *Thermodynamics.*—First law: "In any thermodynamic change a definite amount of heat is expended or goes out of existence for every unit of mechanical work done, and *vice versa.*"

Second law: "It is impossible for a self-acting machine, working entirely independently of any external agency, to convey heat from one body to another at a higher temperature." The foregoing is the form in which the second law of thermodynamics is usually stated. This law has been discussed and restated by Lewis and Randall as follows: "Every system which is left to itself will, on the average, change toward a condition of maximum probability. This law, which is true for *average* changes in any system, is also true for any changes in a system of many molecules."

8. *Gas Laws.*—Statements of the gas laws will be found in the chapter on Respiration.

CHAPTER II

SOLUTIONS

As is pointed out later, living matter is in the form of a solution. It is therefore subject to the conditions and laws which apply to solutions generally. It is desirable to consider briefly some of the forms and properties of solutions in order to understand the activities and limitations of living matter.

A *solution* may be defined as a homogeneous mixture of a gaseous, liquid, or solid substance, called a *solute*, with another substance called a *solvent*. In most solutions the solvent is a liquid, and the molecules of both solute and solvent have great freedom of movement. Many instances are known, however, in which the molecules of one solid are so evenly and intimately distributed among the molecules of another solid, as to take on the general characters of a solution, even though the molecules have very little freedom of movement. Such mixtures are called *solid solutions*. It does not seem essential here to enter into any discussion as to the particular relations which may exist between the molecules of solvent and solute.

Water the Principal Solvent.—In the solutions which will be dealt with here water is the principal solvent. It is the one universal solvent. It is not only the chief constituent of living organisms, but it is as well the solvent and carrier of the chief food and excretory products. In it, in the animal body, are dissolved gases; inorganic salts; a great variety of organic compounds, including carbohydrates and proteins; products of digestion, such as amino acids and simple sugars; and various metabolic wastes. Henderson well states: "Indeed, as clearer ideas of the physical-chemical organization of protoplasm have developed, it has become evident that the organism itself is essentially an aqueous solution in which are spread out colloidal substances of vast complexity."

Water is not only a solvent, but it is itself an electrolyte. A few of the H_2O molecules dissociate into H′ ions and OH^- ions. The number of the molecules so dissociated is relatively very small, and is measured by the concentration of H′ ions or of OH^- ions in the water. At temperatures of 22 or 23°C. this dissociation is sufficient to give 1 gram of weight of free H′ ions in 10,000,000 liters of water, *i.e.*, a solution having a concentration of one ten-millionth normal H′. Inasmuch as there is an equal concentration of OH^- ions in the solution, the liquid will be neutral in reaction. And since there is this dissociation of molecules, water may be spoken of as an electrolyte.

Water seems also to aid in the dissociation of the molecules of many substances which may be dissolved in it. Such substances are spoken of as electrolytes, because they will conduct an electric current.

Other properties possessed by water which render it valuable as a component of living matter are its:

High surface tension, exceeded only by that of mercury.
Low internal friction, resulting in low viscosity.
Great heat capacity.
High heat conductivity.
Latent heat.
Greatest density at 4.0°C.

Classification of Solutions.—Solutions vary considerably in their characters according to the state of the solute relative to that of the solvent. It is customary to classify solutions in two main categories, crystalloidal and colloidal.

Crystalloidal solutions are solutions containing substances in the molecular state (or ionic). These are substances which diffuse readily through animal membranes, lower the freezing point of the solvent, and are generally capable of being crystallized out of their aqueous solutions. Of these substances there are two main groups:

Non-electrolytes, substances the molecules of which, dispersed through the solvent, do not ionize in solution, especially aqueous solution. They, therefore, do not conduct an electrical current. They are chiefly of organic origin. Sugars and starches are well-known examples.

Electrolytes, substances a portion of whose dispersed molecules separate into ions, and which may consequently conduct an electrical current.

Members of either the non-electrolyte or the electrolyte group may combine with the molecules of water so as to form hydrates.

Colloidal solutions are substances of a gelatinous nature, permeable by crystalloidal solutions, diffusing not at all or only very slowly through animal or vegetable membranes, and only slightly affecting by their presence the freezing point or vapor tension of their solutions; also any non-gelatinous substances exhibiting the above properties. Colloidal solutions which are of special interest to the biologist are the following:

Sols are two-phase systems in which multimolecular aggregates of solid substance, constituting a *dispersed phase*, are distributed evenly through the solvent, commonly spoken of as the *continuous phase*.

The *suspension* differs from the sol chiefly in the fact that the particles held in the liquid are of larger and even of measurable size.

The *emulsion* is a colloidal suspension in which the minute particles of the dispersed phase are minute droplets of liquid which is immiscible with the liquid of the continuous phase. These droplets may be of ultramicroscopic size, or they may be of considerable magnitude.

In a *gel* there is a reduction of the amount of solvent relative to the amount of the solid substance, with the result that the substance becomes more viscous than when in the sol condition.

It should be kept in mind that the distinction between crystalloids and colloids is one of condition, not of kind. And, as will be seen presently, the distinction between the crystalloidal condition and the colloidal condition is one not always easy to make.

Electrolytic Solutions.—It has been found that acids, bases, and salts, when dissolved in water, have the power to conduct an electrical current, while certain other substances—for example, sugar—fail to do so. It has also been found that the substances which conduct the electrical current when in solution exert, for the same molecular concentration, a higher osmotic pressure than substances which do not conduct the electric current.

Dissociation.—It was suggested by Arrhenius that substances which conduct the electric current do so by virtue of the fact that in solution there is a splitting of their molecules into two or more portions, atoms, which, becoming associated with a number of molecules of water, are called *ions*. These then behave, so far as diluting the solvent is concerned, like molecules. These ions are of two sorts: those bearing positive charges, the cations, and those bearing negative charges, the anions. The names of the ions receive their respective prefixes from the fact that if electrodes are placed in a solution of an electrolyte and a current is passed through the circuit, it is found that the ions bearing the positive charges, such as H^+, Na^+, Ca^{++}, etc., collect and even give up their charges at the cathode, and those bearing the negative charges, such as Cl^-, SO_4^{--}, CO_3^{--}, etc., at the anode. From what has just been said, it will appear that in a solution of an electrolyte there are present three varieties of particles—positive ions, or cations; negative ions, or anions; and undissociated molecules. The degree to which the dissociation may take place is, of course, a variable, and depends upon a variety of factors, such as the nature of the substance and its concentration in the solution.

Dr. Lewis Jones has given a very vivid picture of the processes which go on in an electrolytic solution when an electric current is passing. He likens the molecules in solution to dancing couples on the floor of a ballroom. Here and there couples are separated and the isolated individuals are moving about by themselves. Suppose a mirror at one end of the room and a buffet at the other, the ladies will gradually accumulate around the mirror and the gentlemen around the buffet. Moreover, the dancing couples will gradually be dissociated to follow this movement.

While Dr. Jones' example gives a good picture, it should not be taken too literally, for not all dissociations are of the same type. There may

be salts behaving as does KCl, which dissociates in such a way that each ion bears a single charge:

$$K^+Cl^- \rightleftharpoons K^+ + Cl^-$$

Such a condition as takes place in the dissociation of $MgSO_4$, may be found, in which each ion bears two charges:

$$Mg^{++}SO_4^{--} \rightleftharpoons Mg^{++} + SO_4^{--}$$

The condition also occurs in which a molecule may dissociate into one ion bearing two charges and two ions each singly charged:

$$Ca^{++}Cl_2^{- -} \rightleftharpoons Ca^{++} + 2Cl^-$$

Similarly, a salt may dissociate so as to give one triply charged ion and three singly charged ions:

$$K_3^{+++}FeCy_6 \rightleftharpoons 3K^+ + Fe(CN)_6^{---}$$

Also various combinations of doubly and triply charged ions occur.

It is importance for the student of physiology to remember that:

All acids give a free H^+ ion.
All bases give a free OH^- ion.
All sodium salts give a free Na^+ ion.
All potassium salts give a free K^+ ion.
All calcium salts give a free Ca^{++} ion.
All ammonium salts give a free NH_4^+ ion.
All nitrates give a free NO_3^- ion.
All chlorides give a free Cl^- ion.
All sulphates give a free SO_4^{--} ion.

Degree of Dissociation.—The degree to which any electrolyte dissociates in solution depends very largely upon the degree to which the solution is diluted. For example, at infinite dilution such a salt as KCl would be completely dissociated so that there would be twice as many particles in solution as there were molecules originally introduced. The solutions which the physiologist uses, however, are not infinitely dilute—and do not contain completely dissociated salts. The dissolved salt molecules are, in general, dissociated to about 86 per cent.

The ionic condition is of great importance in living matter, because in the living substance so many different substances are brought into close relations. There is great opportunity for a vast number of new ionic combinations to be formed. The formation of these new combinations is a part of the normal metabolic activity of every living organism.

Colloids.—For the sake of clearness in the discussion to follow, a few of the more common terms of colloid chemistry should be mentioned. Colloid chemistry deals with a wide range of material things. When any portion of the physical universe, large or small, is set apart so that the actions and reactions which take place within it may be studied, it is spoken of as a *system*. Two main classes of systems are recognized: (1) *homogeneous systems*, those in which a given system is alike in all its parts;

(2) *heterogeneous systems*, those which are composed of dissimilar parts. The systems which are of more particular concern here are of the latter type. They are frequently said to be composed of differing phases, *i.e.*, portions which are mechanically separable. For example. consider a system of ice and water, the ice forming one phase and the water another, and each homogeneous in itself. Together they form a heterogeneous system.

In many cases minute particles of one phase of the systems are scattered throughout the extent of another phase. Such systems are spoken of as *dispersed systems*. If the particles are solid structures of comparatively large size scattered through a mass of liquid, the system is spoken of as a *suspension*. If the particles be of very small, almost ultra-microscopic size, the system is referred to as a *colloidal sol*.

It was pointed out by Thomas Graham (1861–1864) that there are many substances which must, because of their special characteristics, be distinguished from electrolytes and non-electrolytes as these terms are ordinarily used. Among them are those substances now known as colloids.

The distinction made between crystalloids, substances which crystallize out of aqueous solution, and colloids, substances which do not so crystallize, may again be briefly indicated as follows:

Crystalloids	Colloids
Readily obtained in crystalline form from their aqueous solutions.	Composed of two phases, a continuous and a dispersed, immiscible with each other.
	Are not readily crystallized, though some, as albumin and hæmoglobin and the alkaline salts of the fatty acids, may be obtained in beautiful crystalline form.
Diffuse readily in solution.	Do not diffuse readily in solution.
Pass readily through membranes of both plant and animal origin.	Do not readily pass through membranes of plant and animal origin.

The distinction between these two great classes of conditions is in some cases somewhat arbitrary, as no sharp line of demarcation can be drawn between them.

The term *colloid*, as first used by Graham, referred to a definite class of substances like glue and the gums; indeed, the name indicates the similarity found in the behavior of the glue-like substances and those included under the term colloid. More recently the term has taken on a broader significance and is used as referring to a condition of matter rather than to a class of substances. Many colloids appear in the form of solution. It should be kept in mind that a colloidal solution is not necessarily a solution of a colloid, but a solution which exhibits the properties of gummy substances. The substance under consideration may

lie entirely outside the class to which the term colloid was originally given.

Physical States of Matter and Their Combinations.—It is recognized that matter exists in three states or conditions—solid, liquid, and gaseous—also that it is possible to disperse molecules or small particles of a substance in any one of these three states throughout a mass of matter existing in either of the other states. When molecules of a solid, for example, are dispersed through a liquid the result is a solution.

It often occurs that matter is less finely divided than into molecular form, and these minute particles of one or another state (referred to as the dispersed phase) become dispersed throughout the mass or matter of a substance immiscible with it (called the continuous phase). Table 2 gives, in brief, a suggestion of the way in which the different phases may be combined and something of the nature of the resulting combination.

TABLE 2

Condition of dispersed phase	Condition of continuous phase	Resulting condition
Gas	Liquid	Foam
Liquid	Gas	Fog
Liquid	Liquid	Emulsion
Liquid	Solid	Jelly
Solid	Liquid	Colloidal sol
Solid	Solid	Such solids as ruby glass

The combinations of phases which are of a special interest to the biologist are emulsions, jellies, and also such arrangements as gases and solids in liquids. It is certain that studies upon colloids of the latter types can give much important information concerning the general properties of colloidal materials.

Heterogeneous Systems.—All the systems included under the term colloid are heterogeneous in character, *i.e.*, they are composed of unlike portions, thrown into most intimate relations. It is to be expected that such systems as these, in which there are thrown together substances of unlike character and between which there is the possibility of physical or chemical reaction, will be mechanisms in which movements and rearrangements of the constituent particles will be continually taking place. Such systems provide for constant change, and as they provide for instability (within limits) it is evident that they can provide for such displays of energy and material transformations as are seen to occur in matter which is commonly called living.

Sols and Gels.—Colloids exist in two well-recognized conditions, the sol and the gel. These conditions differ from each other especially in the degree of freedom of movement which the colloidal particles exhibit with

respect to each other. The *sols* are, in general, substances which have their solid substance subdivided within a liquid into such relatively large particles that they are able to pass through animal or plant membranes only very slowly, if at all. The substances which form the dispersed phases of sols do not readily go into suspension, and the solutions formed are always very dilute, being rarely more than 1 per cent in strength. Sols frequently show a false fluorescence, and in many instances the colors exhibited by the sols have been held to be dependent upon the sizes of the particles present. This suggestion has recently been called in question.

The *gel* condition is one in which there is an exaggerated resistance to the movements of the colloidal particles with respect to each other. The internal resistance or friction of the system comes to be very high, because of the greater or less degree in which there is a separation from the colloid of the more liquid phase or solvent in which it is dispersed. Many of the inorganic colloids separate from their solutions in an almost water-free condition; others, some inorganic and most organic colloids, retain a large quantity of water upon separation. As examples may be mentioned the gel of colloidal silver, which contains very little water, and gelatine, which, in the gel condition, holds bound a large proportion of water. In general, the change from the sol to the gel condition among inorganic colloids is irreversible; among colloids of organic substances the change from the sol to the gel condition may be repeatedly reversible.

Suspensions and Suspensoids.—Suspensions and suspensoids consist of minute particles of some substance floating in a liquid. The small particles of a suspension may be dispersed throughout the liquid of the continuous phase, may separate from it through the action of gravity or by centrifugal force, and may be redispersed again and again. When allowed to stand, the suspended particles gradually settle out and form a layer at the bottom. The time required for the separation of the particles depends upon their specific gravity, their size, and the viscosity of the liquid, large particles settling quite rapidly while very small ones, near the limits of microscopical visibility, settle very slowly. For example, quartz particles, having a diameter of 0.5 micron or less, do not form a sediment, while larger particles may either spontaneously settle out or may be filtered out.

The suspensoid differs from the suspension in the size of the particles and in the fact that the particles of the suspensoid remain in suspension in the liquid by virtue of the electrical charges which they bear. All the particles of a given suspensoid bear electrical charges of the same sign, and so tend to repell each other in all directions as they move about in the liquid. By these repulsions they maintain their dispersion throughout the liquid. The removal of the electrical charges from the particles (through the passage of an electrical current, the addition of ions bearing

opposite charges, or the addition of colloids bearing charges of the opposite sign) results in either the sedimentation of the particles of the colloidal or the formation of a jelly-like mass.

All suspensions and suspensoids show low viscosity, about that of water, and the temperature coefficient of the viscosity of such liquids is about the same as the temperature coefficient of the viscosity of water. The particles of the suspensoid are in very active movement, the rate of motion being, in part at least, a function of the size of the particles.

Brownian Movement.—As far back as 1827 Dr. Brown, a botanist, noted a movement of microscopical particles suspended in a liquid. This movement consists in an oscillating motion of the particles about a central position, and an erratic translatory motion. The movement is hard to describe but can be seen well in a suspension of very fine particles, such as gamboge, carmine, or India ink in water, under the magnification of the ordinary high powers of the microscope. It is frequently possible to see such movements of particles in living cells. Observation has shown the larger particles in a suspension move slowly and through small arcs or not at all, while the smaller particles exhibit considerable migratory movements. The study of these movements received a great advance upon the invention of the ultra-microscope, when it was seen that the very minute particles revealed by the use of this instrument showed movements of such rapidity and extent as to make observers wonder whether they were not different in kind as well as in degree from the Brownian movements which had been known previous to that time. The problem of the nature of the movements was attacked by new experimental methods and by mathematical treatments, with the result that it now seems assured that the Brownian movements, so called, and the movements of the more minute particles of colloidal solutions are of one and the same kind and are governed by the same laws. The movements are unquestionably due to the impacts of molecules and ions upon the particles. The motion is conditioned by the temperature and viscosity of the liquid; it is inversely proportional to the size of the particles and independent of their electrical charge. Mass appears to have little or nothing to do with their activity. The movements of the particles can be treated as the movements of exceedingly large molecules, with extremely great molecular weights—of the order of 1000 millions in the case of the mastic particles investigated by Perrin.

Physical Properties of Emulsions and Emulsoids.—Most emulsions and emulsoids are of organic origin. These differ from suspensoids in that both phases, the dispersed and the dispersing, are liquid (sometimes of quite high viscosity, like a jelly). Sols which resemble emulsions in showing markedly high viscosity are termed *emulsoids*. Usually the liquids comprising the two phases of the emulsion are immiscible in each other. Gelatine and water form such a system.

Figures 1 and 2 show photographs of two very similar emulsions. Figure 1 shows the appearance of a small drop of cream when viewed under the microscope by means of a dark-field illumination. It will be noted that the small drops of butter fat adhere rather closely to each other, though they are surrounded by the continuous water phase of the emulsion. In Fig. 2 there is a similar representation of a thin film of butter. In this case, however, the minute droplets are water droplets and the continuous phase is the butter fat. In some instances there seem to be mixtures of the two substances in each phase, a concentrated dispersed phase and a more dilute continuous phase, as, for example, a dispersed phase consisting of a small amount of water held in a relatively large amount of protein, and a continuous phase consisting of a small amount of protein in a relatively large amount of water. Among emulsions, both the sol and the gel conditions are found.

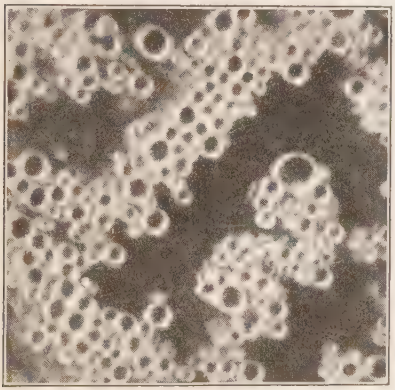


FIG. 1.—An emulsion of oil in water (cream) photographed by means of dark-field illumination. × 525.

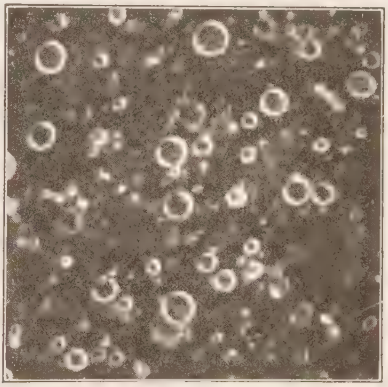


FIG. 2.—An emulsion of water in oil (butter) photographed by means of dark-field illumination. × 525.

Emulsoids differ from suspensoids in the ease with which they dissolve, going into solution without the employment of any of the special methods which have been found necessary for the formation of the ordinary suspensoids. The emulsoid colloids show marked affinities for water and enter easily into intimate relations with it.

Among emulsoids there are colloids which exhibit three very different sorts of behavior:

1. Some, as gelatine, form solutions above a certain temperature, below which point the sol forms a jelly. Such are readily and rapidly reversible.

2. Some, as albumen, when heated form irreversible coagula, *i.e.*, change to an irreversible gel condition.

3. Some, as gums, starches, and casein, neither set to jellies on cooling nor coagulate on heating.

Adsorption.—The fact that the material comprising the dispersed phase of a colloid is in an extremely finely divided condition makes inevitable extensive surfaces at the boundaries between the dispersed and continuous phases. Any reactions or interactions of a physical or chemical nature which might occur between the two components or phases of the system would naturally take place at these interfacial boundaries. So, it might be expected that, among other phenomena, there would be found the force of surface tension made evident in the behavior of the minute droplets or particles which constitute the dispersed phase. It would be natural that chemical changes should take place at these planes of contact also.

It has become a matter of common experience that some finely divided materials have the power to take out of solution certain dissolved substances, such as gases or stains, and to hold them with great tenacity. It is this principle which is effective in the removal of odors from water or butter by means of charcoal. It was at first stated that the gas or stain was absorbed by the finely divided material, but it has become apparent that this is not a case of absorption in the true sense of the term, but a surface condensation of the gas or stain upon the surfaces of the minute particles. In *absorption* there is an actual infiltration of the absorbed substance throughout the mass of the absorbing substance, so that it might almost be said that the solute is dissolved in the substance of the absorbing body. In *adsorption* the adsorbed material is simply condensed upon the surface of the adsorbing material. This statement does not indicate in any way whether the process is to be considered as a physical or as a chemical phenomenon.

Mathews gives an illustration of adsorption which is of interest in making clear what is meant by the process. With his consent it is given here:

Whenever we put on clothing, we adsorb the clothes. We and our environment are a part of a great system. Any change of the environment produces a change in us, and any change in us produces some change in our environment. In this way we and the environment make what is known as a system, which consists of two constituents or phases, one of these being ourselves. As it has two phases or physically distinct parts, the system is heterogeneous. Every morning when we dress, clothing which has been distributed through our environment—dispersed in the surrounding phase, in other words—concentrates at the surface of our bodies; or, speaking technically, it concentrates at the interphase boundary of the system, so that the concentration of clothing at that boundary becomes greater than in the room about us. This is a process of positive adsorption. And at night the process is reversed—the concentration of clothing in the room becoming greater than at the surface of the body—the cloth-

ing passes out of the interphase, and we have then a case of negative adsorption. And we might go on, if we wished, with this treating of the wearing of clothes as a process of adsorption and express it in a curve or isotherm, showing how the quantity adsorbed is a function of the amount in the room; how it proceeds usually to an equilibrium; how it is greater at low than at high temperatures; that it is reversible and not accompanied by any chemical change in the clothes; that it is specific in that certain kinds of clothes are adsorbed with greater avidity than others; that certain adsorbents (people) adsorb better than others; and finally we could prove that the clothing moved into the surface film in virtue of the second law of thermodynamics, and because of the thermodynamic potential in consonance with the principle of Willard Gibbs. In other words, dressing is in every respect a typical case of adsorption, and if we wished to go farther we could actually prove, at least as well and to just the same degree as similar things are proved for the protozoa and other living things so minute that we cannot easily see just what is happening, that the wearing of clothes is a surface-tension phenomenon, and has a purely mechanical explanation. Human behavior, in the wearing of clothes, would thus be explained to the satisfaction of the mechanistic philosopher, and in the same way as the wearing of mineral clothing, called shells, by protozoa has been explained. (This explanation might not be equally satisfying to all students of biology, however.)

The adsorption of one substance upon another is, so far as the physiologist is concerned, to be looked upon both as a result and as a means to an end. It is the result of one or more of several forces. These may be physical, including surface tension, electrical, magnetic, or gravitational force, or molecular cohesion; or the force of chemical attraction. It is a means to an end, in that through the very close associations of molecules brought about it provides the conditions necessary for very significant chemical reactions.

Mathews has pointed out that the chief force involved in adsorption is that force known as cohesion, that force by which the particles of a body are united through the mass, whether they be like or unlike. Two factors influence cohesion: first, gravitational mass; and, second, chemical affinity or chemical mass. Of these, Mathews considers the first the more important. The degree to which one substance may be adsorbed is correlated with the ease with which the substance is condensed, *i.e.*, with the cohesive force which it exhibits. The real difference between solids, liquids, and gases is in the degree of cohesion existing between the molecules, and in the degree to which the molecules depart from the spherical form. This cohesion is greatest in the case of solids and least in gases. Solids whose molecules depart most widely from the spherical form have the greater cohesion—and also the higher melting points. It is this fact, that solids have high cohesion, which enables them to condense other substances upon their surfaces.

Substances in the colloidal condition offer the greatest possibilities for adsorptions to occur, because of the remarkable development of

the interfaces between the continuous and the discontinuous phases. Particles upon which adsorptions take place must be as large as that of the smallest colloidal particles, namely, 1×10^{-7} centimeter in diameter. In all living matter enormous surfaces bounding the different phases are developed. Separations or condensations of material may occur at the interfaces between the dispersed and continuous phases of protoplasm, and at the surfaces of oil drops, of carbohydrate or mineral deposits, of all particles or granules, chromosomes, mitochondria, filaments, etc., and of all cells. If these condensations are colloidal in character, membranes may be produced; if they are crystalloidal, they may take the form of deposits of salts, spicules, shells, bone, etc.

Mathews has also pictured the conditions existing at the surface of a solid or liquid, as follows:

> The amount of cohesion in a molecule may be represented by a certain number of lines of cohesional force radiating in all directions from the center. The molecule may be pictured as cubical. The same number of lines of force leave

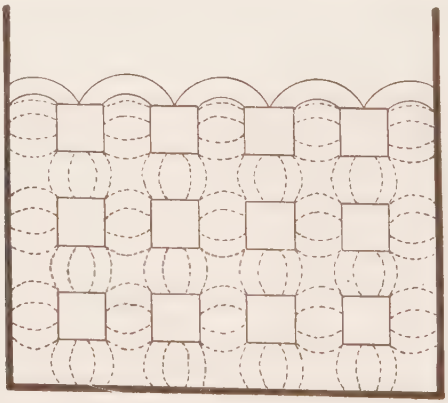


Fig. 3.—Mathews' figure of cubical molecules with four lines of cohesional force reaching outward from each face of the cube. For further description see the text.

each face of the cube. These lines of force go only as far as the next layer of molecules. They must be neutralized or saturated there, just as the electrical lines of force are saturated by the surface upon which they abut. Each species of molecule has a definite power of neutralizing the cohesional lines of force, and it can neutralize just as many lines as it sends out and no more. At the surface of a liquid or solid, the outwardly directed lines of force of the surface molecules have in a vacuum nothing nearby to saturate them, as there are no molecules lying close beyond them and they must abut on something. Consequently, they bend over and abut on the exterior surface of the molecules lying next to them in the surface, as shown in Fig. 3. These lines of cohesional force are assumed to repel each other and to contract just as those of electricity, to which they are indeed related, so that they tend to shorten. The result of this is that

the peripheral layers of molecules are attracted in the plane of the surface not only by the ordinary cohesion, but by the additional force of the cohesion which ordinarily is directed outward. This additional tension in the plane of the surface is what is known as surface tension. It puts the surface of a solid under a strain, and in liquids will draw the liquid into the form of a spherical drop if the mass of the liquid is sufficiently small. Now let us suppose that a molecule of a gas approaches the surface of a solid thus constituted. The approaching gas molecule is also surrounded by its lines of cohesional force which tie it to the other gas molecules. The density of these lines of force is, of course, not great. But when the molecule is driven by its kinetic energy close to the solid surface it enters the field of force of these surface molecules, and they in turn enter its field of force. This field varies in intensity in various solids, as we shall see in a moment, but in the case of carbon or charcoal it is very intense; unless the gas molecule is approaching with very great speed so that its elastic rebound will serve to carry it again out of the field, it will stick to the surface and be trapped there, just as the weaker flies stick to fly paper. Each of these molecules so trapped saturates as large a number of the lines of force as it carries itself. The attraction between gas and solid is proportional to the product of the lines of force each carries. But a gas like hydrogen carries very few such lines. Its cohesion is very small. It can saturate only a few of the lines of the carbon and as a result a considerable quantity of molecules of hydrogen will be required to saturate the cohesion of the solid. This means that a great deal of hydrogen can be condensed. It is clear also that as the outward directed lines of force of the peripheral layer of molecules of the solid are gradually released from abutting on the neighboring molecules so that they abut on the overlying gas, the surface strain of the surface layer, in other words, its surface tension, will be reduced. So that what is called surface tension in a liquid or solid is reduced by the adhesion of molecules of the gas or vapor. It is for this reason that the principle of Gibbs can be so easily applied to this process. Actually, the surface tension of the solid is reduced by the condensation of the gas. This however, it will be observed, is a result, not the cause, of the condensation. The molecules are held on the surface not because they reduce the surface tension, as is generally stated, but because they are attracted by their cohesional attractions to the surface. They are held there by the force of cohesion, not by that of surface tension. The reduction of the surface tension is an incidental result of their cohering to the surface.

Combinations between Colloids and Other Substances.—Evidence for the formation of definite chemical compounds between colloids and other substances is beginning to accumulate. Emulsoids are systems which are peculiarly favorable for the display of chemical affinities. It is easily possible for various substances to enter into the emulsoid system, and, upon entering into combination with one or the other component of the system, to upset the balance previously existing. So it is found that such emulsoids as gelatine, casein, etc. have the ability to enter into chemical combinations with various anions and cations, and that the formation of such compounds is conditioned by the hydrogen-ion con-

centration of the system. It has been shown that gelatine, for example, exists in three states. On the acid side of the isoelectric point it is in the form of gelatine acid salts (chlorides, sulphates, etc.); on the alkaline side it is in the form of metal gelatinates (sodium gelatinate, calcium gelatinate, etc.); at the isoelectric point it is in combination with neither ion.

Sizes of Colloidal Particles.—There is probably no sharp line, as to the size, between particles of a suspension which may be separated out by gravity, centrifugal force, or filtering, and the particles of a suspensoid which may not be so separated. As compared with the sizes of molecules of the common electrolytes the particles of a suspensoid are immensely large, while when studied under the highest powers of the microscope they either cannot be seen at all as separate particles or appear at just about the limit of microscopical visibility. There is much evidence to show that the particles of an inorganic sol are each composed of many molecules. For example, it has been shown that the particles of gold in a colloidal sol have a diameter of about 1 micromicron, *i.e.*, one-millionth of a millimeter. Such particles appear to have a molecular weight of 300,000. The molecular weight of gold is 197. The colloidal particles of gold are therefore made up of hundreds or possibly even thousands of molecules. The particles of starch, albumin, etc. are presumably of corresponding size. The accompanying chart (Fig. 1) is an attempt to show in a graphic way the relative sizes of the particles of crystalloidal solutions, colloidal hydrosols, particles in suspension, oil droplets in fine emulsion, etc.

Osmotic Pressure of Colloids. Some of the statements made by earlier writers as to the physical properties of colloids would lead to the belief that they do not exert osmotic pressure. This assumption was due to faulty methods of making determinations and to the fact that with substances of such high molecular weight it is impossible to work with solutions of very great molecular concentration. One difficulty in the way of proper determinations of osmotic pressure was the lack of suitable membranes. G. Malfitino was the first to point out the possibility of using collodion membranes for this purpose. Such membranes may be used for purifying colloidal solutions by dialysis, as they permit the free passage of electrolyte molecules and other crystalloidal substances, but are not permeable for colloids.

One of the reasons that measurements of osmotic pressure of colloids have been so unsatisfactory is that the solutions employed have been of relatively low molecular concentration, *i.e.*, the diffusion pressure of the solvent in the solution differed but little from that in the pure solvent. A molecular solution of hæmoglobin would contain, say, 50,000 grams of hæmoglobin in a liter—a condition not easily realized. A 1 per cent

solution of hæmoglobin would have relatively so few molecules as to make very little impression in modifying the diffusion pressure of water, amounting only to $\frac{1}{5000}$ of 22.4 atmospheres, or 3.4 millimeters pressure of mercury. While rough measurements of the osmotic pressure of col-

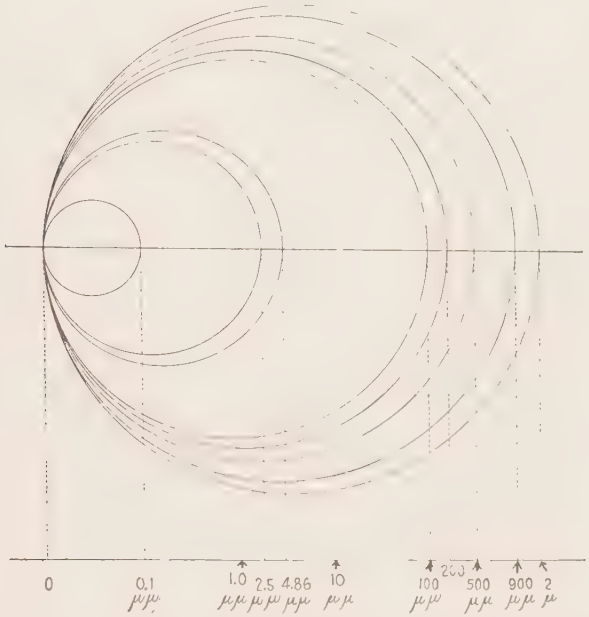


FIG. 4.—This diagram is an attempt to represent the relative size of different materia particles. It is to be noted that, passing from left to right, each scale division has ten times the value of the one immediately preceding it. The circle having the diameter of 0.1 micromicron may represent the maximum size of particles held in crystalloidal solution. The circle having the diameter of 2.5 micromicrons represents the size of the hæmoglobin molecule. The circle having the diameter of 4.86 micromicrons represents the size of salt-free albumin particles. The circle with the diameter of 100 micromicrons represents the upper limit for the size of particles of true hydrosols. The circle having the diameter of 200 micromicrons represents the size of particles just at the border of microscopic visibility. The circle having the diameter of 500 micromicrons represents the upper limit in size of particles of quartz, held in suspension in water, which do not form a sediment. Particles having a larger diameter than this do sediment out. The real form of particles between 200 and 500 micromicrons may be seen with the microscope. The circle having the diameter of 900 micromicrons represents the limit in the size of particles of oil emulsions in water in which separation of oil from water does not take place. Oil particles having a larger diameter than 900 micromicrons will separate out from the water and form a separate layer. The circle having the diameter of 2 microns represents the border between the size of particles which may be filtered out of a suspension and those which may not be separated from the suspension by filtering.

loidal sols may be made in a variety of ways, as by the determination of diffusion constants, depression of the freezing point, elevation of the boiling point, etc., the results are much less accurate than those of direct determination made by the aid of suitable membranes.

The earlier erroneous idea that colloidal solutions exert no osmotic pressure was very likely due to the fact that the solutions measured were always very dilute, in many cases impure, and that the methods employed were not sufficiently refined. The collodion membranes employed by R. S. Lillie, J. Loeb, and others obviate the most serious difficulties. Lillie's method consists in the formation of a collodion film on the inside of a test tube or flask, and the utilization of this membrane. The membrane is formed by introducing into the tube or flask a concentrated solution of collodion, either filling the flask to the top and then emptying out the excess collodion or putting in a smaller amount of the collodion and then rotating the flask until its whole inner surface is coated. The excess is drained off. After drying for a few minutes in the air the film can easily be separated from the wall of the flask. The collodion sac is now bound tightly to a rubber stopper through which passes a glass tube which may serve as a manometer. The collodion tube permits electrolytes and other crystalloids to pass freely but is impermeable to colloidal substances.

Molecular Weights of Colloids.—The emulsion colloids of special interest to the physiologist have been shown to have very high molecular weights. The figures given indicate the hopelessness of obtaining solutions of the most important protoplasmic colloids having any considerable concentration. All the solutions which are dealt with must be, from a molecular standpoint, very dilute, and so will show very little osmotic pressure.

TABLE 3

SUBSTANCE	MOLECULAR WEIGHT
Lower achroödextrin	905
Commercial dextrin	2,700– 6,200
Congo red	2,088
Erythrodextrin	3,000– 6,800
Acid dextrin	4,000
Higher achroödextrin	8,200–11,700
Amylodextrin	20,500–22,200

Molecular Weights of Certain Proteins.—The most recent authoritative statement concerning the molecular weight of the proteins is given by Cohn. He has himself measured the molecular weights of some of the proteins, has taken the results of other observers who made use of analytical methods, osmotic-pressure methods, etc., and has given a statement of the probable molecular weights in figures which seem to harmonize the hitherto conflicting statements.

TABLE 4

Name	Molecular weight by analytical methods	Molecular weight by osmotic pressure	Probable molecular weight
Egg albumin	33,800	34,000	33,800
Serum albumin	5,000	45,000	45,000– 50,000
Bence-Jones Protein	24,500	.....	49,000
Haemoglobin	16,700	48,000	50,000– 65,000
Edestin	29,000	.....	58,000
Haemocyanin Limulus	22,700	80,000	68,100
Pseudoglobulin	27,000	.....	81,000
Euglobulin	27,000	.....	135,000
Gelatine	10,300	.....	123,600–164,800
Fibrin	42,000	.....	168,000
Casein	12,800	.....	192,000

Colloids in Physiology.—The colloidal condition is a matter of extreme interest to the biologist, for all living matter is in the colloidal condition. Indeed, it seems impossible to conceive of living matter as existing in any other condition than one which provides for complexity and stability amidst constant change.

In metallic hydrosols there exist two phases, the continuous and the dispersed. Each of these is simple and homogeneous in itself. Emulsions and emulsoids still involve, in many cases, two simple immiscible phases. Living matter is a colloid which is much more complex. It has its own continuous and dispersed phases, each of which may be a liquid. One is usually more liquid than the other. There is frequently found a change from the sol to the gel, involving a variation in the water content of the colloid. Each of the phases of the colloid may be complex and heterogeneous in character. Undoubtedly, the colloids form the stable substrate of the living substance. Associated with the colloidal elements are certain crystalloidal substances which penetrate to all parts of the colloidal complex, and which here and there become temporarily bound up in the complex—and are then freed to become again, at some other point, a part of the living machine. Some of the phenomena which are called *vital* are beyond question the direct result of the shifting here and there of molecules of one sort or another in the colloidal complex.

The colloids which have a special interest for the physiologist may be grouped under three general heads:

1. Carbohydrates.
2. Lipoids and fats.
3. Proteins.

These classes of substances demand some consideration because they are the products of the activity of living cells and also because, as food

for the living animal, they furnish the energy needed for protoplasmic activity and the materials for the replacement of worn-out protoplasm and for the construction of new living substance.

1. *Carbohydrates.*—The members of this group of substances occur in both the crystalline and the colloidal condition. They consist of carbon, hydrogen, and oxygen in the proportions of $C_n(H_2O)_{n-1}$. The value of n is rarely if ever less than 30 and may rise to 200 or more. According to the amyloin theory, the starch molecule is composed of five amylin groups each having the formula $(C_{16}H_{20}O_{10})_{20}$. Under the action of diastase one after the other of these groups is separated from the parent molecule and is then split into its simpler components. The last remaining group is known as stable dextrin. All of these groups are subject to further splitting and give rise finally to maltose. While the starches are the most commonly distributed organic substances found among plants, and therefore form a very large and important part of the food of animals, only one example of this group of substances, *viz.*, glycogen, is found in animals. Starch occurs in the form of white granules of microscopic size, insoluble in water, but if heated the granules swell and form with the water an opalescent paste.

Glycogen.—Glycogen is an animal starch found in all the growing cells of the animal body, and is especially abundant in higher animals in the liver and muscles. It also occurs abundantly in the digestive glands of oysters. It is an amorphus compound, white in color, and is readily soluble in hot water.

Dextrin.—The dextrins are really cleavage products of the starches, transition stages between the starches and the sugars, but still have molecules so large as to render their solutions colloidal in character. They are amorphous substances, readily soluble in water, in which they form thick, sticky solutions. The ordinary library paste forms a familiar example. Three forms of the dextrins are commonly recognized:

Amylodextrin (soluble starch), readily soluble in hot water, slightly soluble in cold water, and gives a blue color when tested with iodine.

Erythrodextrin, easily soluble in hot water, and gives a reddish color with iodine.

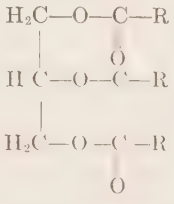
Achroödextrin, easily soluble in hot water, has a sweetish taste, and gives no color with iodine.

Cellulose.—This substance, while very abundant in plants, rarely occurs among animals. It is to be found, for example, in the tests of the Tunicates. The substance has a negative importance to animals because of its general indigestibility. Many forms of animals are entirely unable to digest it by means of their digestive enzymes, though their alimentary tracts may contain bacteria which can effect its decomposition into carbon dioxide and marsh gas.

Gums and Pentosans.—The gums, of which gum arabic and gum tragacanth may be examples, are substances produced by plants; they are of a translucent, amorphous character. Some of them form clear solutions in water; others merely swell up into a glutinous mass. On account of the difficulty with which they may be hydrolized, they have but little food value. Most animals are unable to digest them.

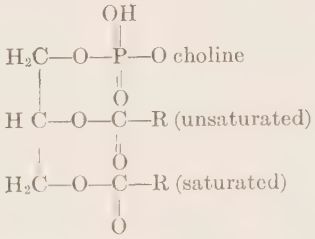
2. *Lipoids and Fats.*—The fats are the glycerides, *i.e.*, glycerole esters of the organic acids. By some authors the term *lipoid* is used as a collective term including all fatty substances. The better usage, however, appears to be to use the term for substances of a fat-like nature which are found in small amounts in animals and plants which, when hydrolyzed, yield fatty acids or their derivatives and some other group.

The fats are constructed upon the general formula



These substances are not soluble in water nor in aqueous solutions, but may be readily emulsified by shaking with alkalies or solutions of bile salts.

The lipoids occur almost universally in plant and animal tissues, and occur especially in those parts in which vital activities are most emphasized. Just what rôle these substances may play in life phenomena is not known. It has been thought that they may have some important function to perform in connection with the maintenance of a cell membrane suitable for absorption. In the molecule of lecithin, which may be taken as an example of the lipoids, one of the fatty-acid radicals is replaced by a phosphoric-acid-cholin radical, thus:



This substance emulsifies spontaneously in water, then the particles swell and form a turbid colloidal solution.

3. *The Proteins.* – These are the most important and most characteristic components of living matter. No living matter is known which does not contain them. They differ from the carbohydrates, fats, and

lipoids in the facts that the molecules of most of them are much larger and more complex in their arrangement, and that nitrogen forms a constituent part of the molecule. The average percentage composition of proteins is as follows:

	Per Cent
Carbon	50–55
Hydrogen	6.5–7.3
Nitrogen	15–17.6
Oxygen	19–24
Sulphur	0.3–2.4

Other elements, *e.g.*, phosphorus, may be included in small amounts.

The size of the protein molecule is always very large, since it is built up by the linkage of a number of relatively simple substances known as amino acids. About twenty different amino acids are now known, and as these are combined in varying numbers, proportions, and relations to each other it will be apparent that the possible number of combinations which may be formed is almost beyond calculation. The various amino acids are linked in the protein by the joining of the COOH group of one to an NH_2 group of another, with the elimination of water, thus CO[OH H]HN. Some COOH and NH_2 groups are left uncombined.

Physical Properties of the Proteins.—The proteins as a group show the following physical properties:

1. They have low power of diffusion and dialysis through animal and plant membranes.

2. Most proteins which are in colloidal solution coagulate upon heating. Proteins not in solution lose their power to swell and become *denatured*. The coagulation temperature of different proteins was for a long time held to be specific and diagnostic, but this idea has been shown not to be well founded. The coagulation temperature of a given protein may vary within wide limits, conditioned by the salt content of the solution, or even more by the hydrogen-ion concentration of the solution. For example, a 1 per cent solution of crystallized egg albumen at pH 4.8 precipitates (forms coagulum) at about 60°C., while at pH 4.39 the coagulation forms at about 80°C. At pH 4.25 or below, heat coagulation no longer occurs at 95°C., the solution merely becoming opalescent. Coagulation due to heat and to the action of the salts of the heavy metals is irreversible.

3. Proteins are insoluble in strong salt solutions. This condition is unlike the coagulation by heat, inasmuch as the dilution of the solution causes the protein to redissolve, while a protein coagulated by heat cannot be thus redissolved. This salting-out process, as it is termed, is frequently used in the separation of one protein from another.

4. Some proteins—for example, egg albumen, horse albumin, hæmoglobin—have been crystallized out of solution. The forms of the crystals

are fairly characteristic for these substances, but vary somewhat with the salt content of the fluid from which they are deposited.

Chemical Properties of the Proteins.—Colloid chemists have until recently believed that the ultimate unit of the colloidal solution is not the ion or the molecule but an aggregate of molecules. It was considered unlikely that the commonly accepted views of chemists in regard to the mathematical expression of combinations between various substances could be valid with such substances as the proteins. It had been recognized that some form of union between proteins and other substances does take place, but it was thought to be of a physical rather than of a chemical nature. Such combinations were thought to be adsorptions—merely surface phenomena. In fact, it was not for a long time recognized that even adsorptions might be chemical combinations rather than superficial condensations of one substance upon particles of another. More recent investigations show that protein solutions contain protein molecules and ions just as do other solutions, and that these molecules and ions have powers of combination with other molecules and ions not at all unlike those of other substances whose behavior has been better understood.

Proteins, because they contain both basic (NH_2) and acid (COOH) groups, are considered *amphoteric electrolytes*, capable of forming ionizable salts with acids as well as with alkalies, according to the hydrogen-ion concentration of the solution in which they are placed. They exist, therefore, in three conditions:

1. They form salts with acids, as gelatine chloride. This occurs when the hydrogen-ion concentration exceeds a certain critical value, in this case pH 4.7.

2. They form salts with bases, as sodium gelatinate. This action takes place when the hydrogen-ion concentration is below the critical value.

3. At the critical value of the hydrogen-ion concentration the protein is not able to combine with either an acid, a base, or a neutral salt. This critical hydrogen-ion concentration is called the *isoelectric point* of the protein.

Among the chemical properties of the proteins of importance to the physiologist should be mentioned their general color reactions. These color reactions are due to a reaction between some one or more of the constituent groups of the complex protein molecule and the chemical reagent used in the particular test. Inasmuch as not all proteins contain all of the same groups, it is found that the tests vary in intensity of reaction. In case of any doubt it is always wise to submit the sample under examination to more than one of the recognized tests before making a decision as to the nature of the substance.

Color Tests.—Some of the more common color tests are the following:

1. *The Biuret Reaction.* The addition of two to five drops of a dilute solution of copper sulphate to solutions of proteins made alkaline by the addition of strong sodium or potassium hydroxide results in a purplish violet or pinkish violet. The depth of the color is dependent upon the nature of the protein present. This reaction depends upon the presence of two or more $CONH_2$ groups, either joined directly or through a single atom of carbon or nitrogen.

2. *Xanthroproteic Reaction.*—This is the reaction caused by nitric acid whenever it comes into contact with animal tissue. The yellow coloration is caused by the action of nitric acid upon the phenyl group. This group occurs in tyrosine, phenylanaline, and tryptophane. The color is intensified by heating and is changed to orange red by the addition of ammonia.

3. *Millon's Reaction.*—This reaction is especially valuable for testing solid substances for proteins, in which case the reagent is added directly to the solid substance and boiled. The protein gives a red color. In the case of solutions of proteins which are not precipitated a red color of the solution is indicative of the presence of protein. The reaction is due to the presence of tyrosine.

Classification of the Proteins.—The classification of the proteins adopted by the American Physiological Society and the American Society of Biological Chemists is as follows:

I. Simple proteins:
   - Albumins
   - Globulins
   - Gluteins
   - Prolamins
   - Albumenoids
   - Histones
   - Protamines

II. Conjugated proteins:
   - Nucleoproteins
   - Glycoproteins
   - Phosphoproteins
   - Hæmoglobins
   - Lecithoproteins

III. Derived proteins:
   1. Primary protein derivatives:
      - Proteans
      - Meta proteins
      - Coagulated proteins
   2. Secondary protein derivatives:
      - Proteoses
      - Peptones
      - Peptides

The Colloidal Behavior of Proteins.—It has already been stated that proteins are complex substances which contain both COOH and NH_2

groups, and so are able to combine with either acids or alkalies, depending upon the hydrogen-ion concentration of the system, and thus to form either acid or basic salts. Loeb was able to show that there are definite stoichometrical relations between the amounts of acid or base which combine with fixed amounts of protein. Experiments upon the power of gelatine to combine with silver nitrate at different pH values, ranging from $\frac{M}{8}$ to $\frac{M}{8192}$ HCl, showed that on the acid side of the isoelectric point the gelatine occurred in the form of gelatine nitrate, while on the alkaline side of the isoelectric point it was in the form of silver albuminate (see

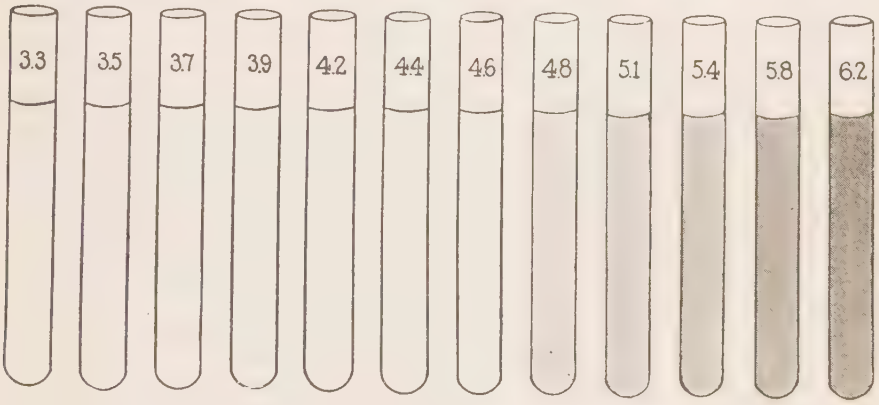


FIG. 5.—Proof that cations combine with proteins only on the alkaline side of the isoelectric point. Powdered gelatine brought to different pH was treated in a dark room with M/64 $AgNO_3$ and then washed with cold water to remove the silver not in combination with gelatine. The gelatine was liquefied, brought to a 1 per cent solution, and the pH was determined. The solutions were then poured into test tubes and exposed to light. In about half an hour the gelatine of pH > 4.7 was dark while the gelatine of pH 4.7 or less remained permanently clear though exposed to light for over a year. The pH of each gelatine solution is marked at the head of each test tube. (*From J. Loeb, "Proteins and the Theory of Colloidal Behavior." By permission of the McGraw-Hill Book Company, Inc.*)

Fig. 5). Similar results were obtained when the gelatine was allowed to react with nickelous chloride, cupric acetate, basic fuchsin, and neutral red, in each of these cases the cation being washed out of the gelatine at pH values less than 4.7, while they remained combined with the gelatine at pH values more than 4.7. It was found that the opposite was true with salts whose anions can be easily detected by simple color tests, such as potassium ferrocyanide, sodium thiocyanate, or acid fuchsin. In all of the experiments mentioned the gelatine failed to combine with either anion or cation at pH 4.7. Similar experiments have been made upon other proteins, such as casein, egg albumen, edestin, serum globulin, etc. In all cases the results were similar. It may, therefore, be considered proved that the proteins are amphoteric electrolytes.

The Donnan Equilibrium.– Donnan has shown that when a membrane separates two solutions of electrolytes, one of which contains one

ion which is not able to diffuse through the membrane, while the others can so diffuse, an unequal distribution of the different ions on the two sides of the membrane results. Dr. Hitchcock has made a very clear statement of this matter:

For example, let GCl represent a soluble chloride ionizing to form G^+ and Cl^-, and let the membrane be impermeable to the ion G^+. Suppose also that HCl is present, and that the H^+ and Cl^- ions are freely diffusible through the membrane. The conditions at equilibrium may be represented as follows, the small letters indicating molar concentrations of the different ionic species:

I	II
$z\ G^+$	
$y\ H^+$	$H^+\ x$
$y + z\ Cl^-$	$Cl^-\ x$

Donnan showed that the relation between the concentrations of the ions must be such that $x^2 = y(y + z)$. This means that the product of the concentrations on one side of the membrane of any two diffusible univalent ions is equal to the product of the concentrations of the same two ions on the other side of the membrane. From the equation it is evident that x and y must be unequal. This unequal distribution of diffusible ions at equilibrium, with the equality of products expressed in the equation just given, constitutes the outstanding feature of the Donnan equilibrium.

If instead of GCl and HCl the electrolytes had been G_2SO_4 and H_2SO_4, the conditions at equilibrium would be as follows:

I	II
$z\ G^+$	
$y\ H^+$	$H^+\ x$
$\frac{y}{2} + \frac{z}{2}\ SO_4^-$	$SO_4^-\ \frac{x}{2}$

Then the relation becomes $x^3 = y^2(y + z)$. This shows that a change in the valence of a diffusible ion changes the degree of the equation. If the non-diffusible ion has a higher valence than 1, then the equation can be retained in the same form by letting z represent the equivalent instead of the molar concentration of the non-diffusible ion.

Associated with the fact that the protein is uncombined with acid or alkali at the isoelectric point it has been shown by Loeb that the isoelectric point is also the point at which occurs the lowest membrane potential, the least swelling of the protein, the lowest viscosity, the lowest osmotic pressure, and the lowest conductivity. The accompanying chart (Fig. 6) illustrates Loeb's findings in regard to these facts. These results obtained from investigations upon gelatine have been found to apply also in the case of other proteins, and may very reasonably be thought to be of quite general application and significance.

There are also properties of the proteins in which their behavior is like that of the crystalloids. In their reactions with acids and bases and in their solubility relations the proteins behave like the amino acids, which are classed as crystalloids. The slight influence of concentration and acidity on the viscosity of egg albumen solutions indicates that in this respect egg albumen behaves like an

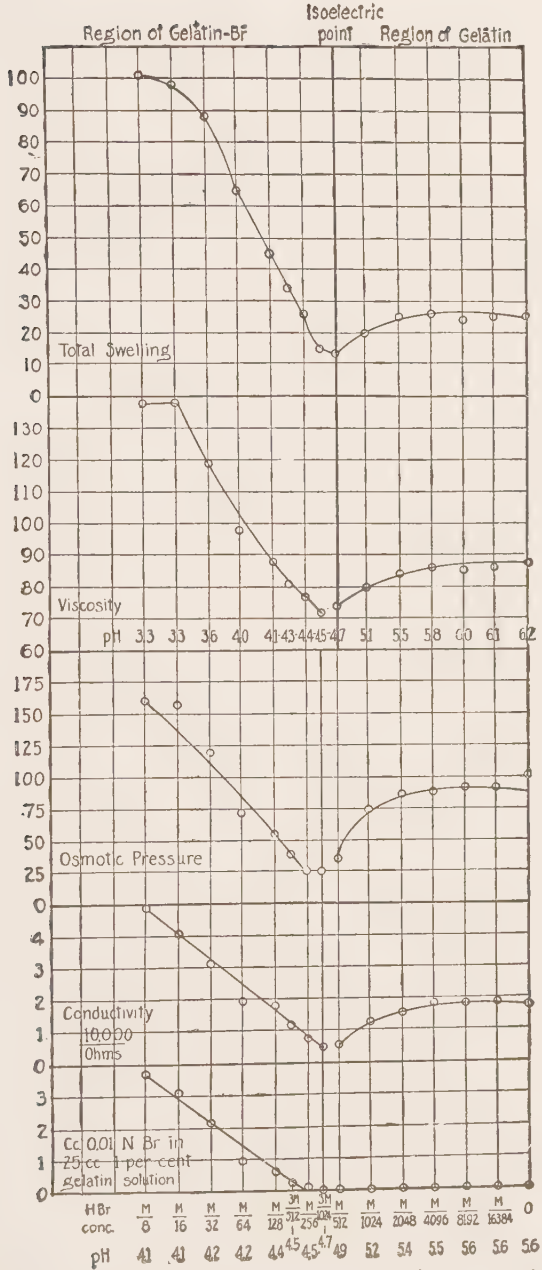

Isoelectric
Region of Gelatin-Br
point
Region of Gelatin
Total Swelling
Viscosity
pH 3.3 3.3 3.6 4.0 4.1 4.3 4.4 4.5 4.7 5.1 5.5 5.8 6.0 6.1 6.2
Osmotic Pressure
Conductivity
10,000
Ohms
Cc 0.01 N Br in
25 cc 1 per cent
gelatin solution
HBr
conc.
M/8 M/16 M/32 M/64 M/128 3M/512 M/256 3M/1024 M/512 M/1024 M/2048 M/4096 M/8192 M/16384 0
pH 4.1 4.1 4.2 4.2 4.4 4.5 4.5 4.7 4.9 5.2 5.4 5.5 5.6 5.6 5.6

FIG. 6.—Showing that the physical properties of gelatine are a minimum at the isoelectric point. (*From J. Loeb, "Proteins and the Theory of Colloidal Behavior." By permission of the McGraw-Hill Book Company, Inc.*)

amino acid. Properties which depend on the forces of cohesion are also found in crystalloids. Such crystalloidal properties do not depend upon the Donnan equilibrium; it is possible that solubility and cohesion may be influenced by the chemical nature of added ions, and this may account in part for certain peculiar results on swelling in high concentration of salts. Such considerations have led Loeb to suggest that the distinction drawn by Graham between colloids and crystalloids or the colloidal and crystalloidal states of matter, should be replaced by the distinction between colloidal and crystalloidal behavior, the former being evident only when the non-diffusibility of an ion through a membrane comes into play.

CHAPTER III

DIFFUSION AND OSMOSIS

The way in which food materials become available for use in the body and the way in which gaseous transfers are brought about depend upon certain purely physical processes which enter largely into these physiological activities.

Diffusion.—It is well known that a gas enclosed within the walls of a container tends to distribute itself equally throughout the container, the molecules of the gas striking away from each other on all sides. The force thus exerted by the molecules in beating away from each other, and finally resulting in the equal distribution of the molecules throughout the whole space occupied by the gas, is spoken of as *diffusion pressure*. It is recognized that the pressure of the gas against the sides of the container is proportional to the amount of gas contained and hence to the number of impacts of gas molecules per unit of surface against the sides of the container.

Further, if two containers holding different gases be placed in communication, there will be a process of diffusion from the one container to the other in such a way that the two gases become equally distributed throughout the whole of the space. Each gas moves from its position of higher concentration, *i.e.*, its greater number of molecules per unit volume, to a position of lower concentration, *i.e.*, lesser number of molecules per unit volume, and the equilibrium is not reached till the partial pressure of each gas is the same throughout the whole space.

The term *diffusion* applies not only to the molecules of a gas becoming equally distributed among the molecules of another gas, but also to the mutual distribution of the molecules of a gas and a liquid, of a liquid and another liquid, or of a liquid and a solid.

In the study of physiology it is found that diffusions of gases, liquids, and solids through other liquids hold a very important place. Without unnecessarily multiplying examples the case of the diffusion of sugar through a liquid may be mentioned. If a few crystals of sugar are placed at the bottom of a jar, covered with a layer of water, and allowed to stand undisturbed, after a little time the crystals of sugar have disappeared from the bottom of the jar, and have gone into solution—have become diffused equally throughout the whole mass of the liquid.

Mutual Interdiffusion. The mutual character of diffusions such as have just been mentioned has been frequently overlooked. This is

especially likely to be true in considering two interdiffusing substances which are present in entirely disproportionate amounts. It is very easy to think and to speak of sugar dissolving or diffusing in water, but it is less customary to think of water molecules diffusing among sugar molecules, when the amount of water present in the system is much greater than that of the sugar. Both solvent and solute possess and exert diffusion pressure.

Membranes.—It now becomes necessary to introduce a new concept — that of a membrane. A membrane has been defined as any thin, soft, pliable sheet or layer of animal or vegetable tissue. As will be seen, such membranes are derived from colloidal structures and are the result of colloidal activity. It may be said that as the amount of water becomes reduced in a layer of colloidal material the diffusion of colloids into and through it becomes lessened in rate and finally stops. A further reduction in the amount of water present in the colloidal layer results in retarded and lessened diffusion of crystalloid substances. If the reduction in permeability be extended to the point where the solvent only (and not any dissolved substance) is able to pass through the membrane, the membrane may be said to be semipermeable.

The definition given above is not sufficiently inclusive, for it does not take into account the possibility of the formation of membranes except by living cells. There are also membranes formed by the action of inorganic chemicals. Some of these have supplied our most valuable information with regard to osmosis and osmotic pressures.

Osmosis.—If the space of a container is divided into two parts *a* and *b* by means of a parchment partition *m* and there is placed on one side of the partition a solution of sugar and on the other side of the partition distilled water, it will be found, after an interval of time, that there has been a diffusion of sugar through the membrane and that it has appeared in the liquid of the other chamber. In this case there has been a flow of sugar from its position of higher concentration through the membrane to a position of lower concentration, just as in the case of the gases which became thoroughly mixed. As with the gases, this flow will continue until there is an equal distribution of the sugar on the two sides of the membrane. There has also been a flow of water molecules through the membrane from the pure water into the solution, due to the difference in the concentration of water molecules per unit volume. This experiment may be further modified by placing in chamber *a* on one side of the membrane a solution of salt and in chamber *b* on the other side of the membrane a solution of sugar. In this case the sugar will flow through the membrane from its position of higher concentration to a position of lower concentration until an equilibrium is reached. In similar manner there will be a flow of salt through the membrane from its position of higher concentration to a position of lower concentration

until salt equilibrium is established on the two sides of the membrane. It will be noted that these two diffusions are taking place in opposite directions through the same membrane at the same time. They do so without in the least interfering with each other. It may, however, be found that the diffusion of one substance takes place at a faster rate than that of the other. This may be due to the quality of the membrane employed or to a difference in diffusion tension or molecular speed. Membranes differ in their permeability to dissolved substances. So it may be found that a certain membrane will be very permeable to salt and more slowly permeable to sugar, or a membrane may be impermeable to both the salt and the sugar, while it is still readily permeable to water. A membrane of this sort would be termed *semipermeable.*

What is Osmotic Pressure?—Among physicists and chemists there has been much disagreement and misunderstanding as to the nature of the force which causes water to pass through a membrane from pure solvent or from a less concentrated solution into one which is more concentrated. The explanation of osmosis and of osmotic pressure given by Van't Hoff and Arrhenius, and refined by other investigators, has been accepted by so large a number of scientists as to give it almost the authority of a classic. A brief statement of this theory must suffice. In the seventh decade of the last century Pfeffer, a botanist, made direct measurements of osmotic pressure. These measurements comprised an important part of the data from which Van't Hoff made his great contribution: that the so-called gas laws apply also to substances in solution, and that a non-electrolytic substance in solution exerts the same osmotic pressure, and otherwise behaves as it would if it were in the form of a gas, at the same temperature, and if it occupied the same volume as the solution. The gas laws mentioned are those of

Boyle.—The volume occupied by a gas varies inversely as the pressure, provided the temperature is kept constant, and of

Gay-Lussac.—The volume occupied by a given mass of gas increases as the temperature is raised, and the relative expansion is the same for all gases.

Van't Hoff was able to show that, within limits, the osmotic pressure of a dilute solution is proportional to the concentration of the solute and to the absolute temperature. He also applied Avagadro's principle (that equal volumes of all gases at the same temperature and pressure contain the same number of molecules) to solutions and found that as a working hypothesis this assumption is in reasonable harmony with the facts. He thus has presented to us the statement that at a given temperature equal volumes of two solutions which have equal osmotic pressures have also equal numbers of molecules.

Those who have succeeded Van't Hoff are by no means agreed as to the real nature of osmotic pressure. The view supported by Van't Hoff

is that osmotic pressure is a real pressure due to the bombardment of the walls of the container—or the surface film of the liquid—by the solute molecules. This idea has been subject to most careful scrutiny, with the result that many now hold to the idea that osmotic pressure is the force occasioned by diffusion of the solvent through a semipermeable membrane, from a less concentrated to a more concentrated solution. This force can be measured by the hydrostatic pressure required to prevent such diffusion. Washburn takes the position that

"osmotic pressure is the pressure difference which must be established upon the solution and pure solvent respectively, in order to make the escaping tendency of the solvent the same from both of them. The osmotic pressure is, therefore, not a real pressure existing within the solution but is a definite physical quantity, quite independent of osmosis, semipermeable membranes, or molecular theory."

Haldane has recently given in mathematical detail and completeness an explanation of the phenomena of diffusion through membranes in such a way as to make it very evident that what is to be dealt with in the so-called cases of osmosis and osmotic pressure is not the movements of the molecules of the solute but the molecules of the solvent. It is not possible in this place to give in detail all of Haldane's explanation, but some of the main features may be pointed out. In the first place, the view may be accepted that, in a gas in a container at atmospheric pressure, the molecules of the gas do not occupy all of the space within the container. Each molecule has a certain size, and if all of the sizes of the molecules contained within the space of a liter of a gas at atmospheric pressure are added together, the volume will be considerably less than a liter. Even if, instead of the actual volume of the molecules, the volume which all of the molecules present may occupy as the result of their molecular movements be taken the volume would still be less than a liter. But in a liquid the conditions are different. The molecules of the liquid are much more closely related to each other and the free space existing between the molecules of the solvent, where it might be possible for solute molecules to find a place, is very much restricted. In the case of water it amounts to only 0.118 of the whole volume occupied by the water. When a solute is dissolved in the water, without any chemical change taking place, it is found that there is always an increase in the volume of the solution. It is customary to say that the solute is being diluted with water, and that the molecules of the solute now in solution will act as they would if they were present in the form of a gas in a container having a capacity equaling the volume of the liquid. This statement of the case is now revised by Haldane. When the solute is dissolved in the water the water is really being diluted by the solute. The fact has just been mentioned that there is between the molecules of the water very little free space in which the molecules of another substance may find

lodgment. So, as sugar molecules are added to the water, not only is the total number of molecules present in a unit volume of the solution increased, but the number of water molecules in a unit volume actually decreased. By reducing the number of water or solvent molecules the diffusion pressure of the water is reduced. Haldane's analysis of the facts shows very clearly that the molecules of the solute should not be thought of as exerting gas pressure in the solution, as has been held for a long time, but that the effect of the solute molecules is simply to reduce the normal diffusion pressure of the water. There is another force, the intermolecular pressure—that pressure which holds the solvent molecules together as a liquid—which has been found to be the same in both solution and solvent.

Reference has been made to the fact that substances soluble in water tend to become equally distributed through the water in which they are dissolved by virtue of the diffusion pressures of both solute and solvent. Reference has also been made to the fact that certain dissolved substances are able to pass through certain membranes as well as through the liquid solvent. If, now, instead of having a membrane which is permeable to the solvent and to the substance dissolved in it—the solute—there is a membrane which is permeable to the solvent but impermeable to the dissolved substance, there will be set up a process known as osmosis, in which the molecules of the solvent are free to pass from one side to the other of the membrane, while the molecules of the solute are retained on one side of the membrane, and not allowed to pass.

In the case previously described it was shown that the molecules of the dissolved substance passed through the permeable membrane from their position of higher concentration to their position of lower concentration, by virtue of the difference in diffusion pressure of the solute on the two sides of the membrane. In the case now under consideration it is not possible for the molecules of the solute to pass. It is perfectly clear, however, that the solution under consideration is less concentrated in water molecules per unit volume than is the pure solvent on the other side of the membrane. In this case the water molecules will pass from their region of higher concentration on the pure solvent side of the membrane, through the membrane, to their position of lower concentration, and will continue so to pass so long as there is an unbalanced diffusion pressure. "*This pressure which drives the solvent through the membrane has been customarily called osmotic pressure and is simply the excess pressure required to equalize the diffusion pressure of the solvent from solution to pure solvent to that from pure solvent to solution.*" This excess varies as the molecular concentration of the pure solvent, the absolute temperature, and the ratio of solute to solvent molecules vary.

It is a very simple matter to show experimentally that the diffusion pressure which causes water to pass through a semipermeable or slowly

permeable membrane from pure water into sugar or salt in solution in water is a real force. A piece of moistened goldbeater's skin or other membrane may be fastened tightly over the funnel end of a thistle tube, making sure that there is no leak in the joint between membrane and glass. Then the funnel and a short distance up the tube may be filled with a sugar solution. Immerse the funnel of the tube in a beaker of distilled water and fasten so that the levels of the liquid inside and outside the tube are the same. Gradually the liquid inside the tube will be seen to rise, and it will continue to do so until the hydrostatic pressure of the liquid inside the tube equals the excess of the diffusion pressure of the pure solvent in the beaker over that of the solvent in the sugar solution within the tube. The height of the liquid column will, when it comes to rest, give a measure of the difference in diffusion pressures, or the osmotic pressure. This last statement is, of course, based upon the assumption that no sugar can escape through the membrane and that the sugar solution remains of the same concentration throughout the whole of the experiment.

Determination of Osmotic Pressure.—The accurate determination of the value of the diffusion pressure exerted by any solvent, although theoretically simple, proves actually to be very difficult. The difficulty of preparing membranes which will hold back all of the molecules of the solute and at the same time remain unbroken by the tremendous pressures against them is almost beyond belief, and the difficulty of maintaining a constant concentration of solution within the chamber of an osmometer calls for most painstaking care and ingenuity.

Fortunately, it is not necessary to depend alone upon direct measurements of osmotic or diffusion pressures in order to know their values. It has been pointed out that there exists a very definite relationship between the diffusion pressure of a solvent and the freezing point of that solvent. If the diffusion pressure of water, for example, is lowered by dilution with solute molecules, it also appears that the freezing point of the solvent is lowered, and the amount of the depression of the freezing point is proportional to the amount of dilution.

The pressure exerted by a gram molecule of a gas at 0° when compressed to the space of 1 liter is 22.4 atmospheres. So also, when there is dissolved 1 gram molecule by weight of solute (of a non-electrolyte) in 1 liter of pure solvent, the osmotic pressure of the solution is 22.4 atmospheres at 0°C. (273° absolute), and the molecular depression of the freezing point of the solution is -1.87°C. (The determinations made upon the freezing points of cane sugar and dextrose solutions indicate that the former, in solutions up to high concentrations and high temperatures, is present as a pentahydrate, and the latter as a dihydrate.)

The osmotic pressure in atmospheres of a solution may, then, be calculated by the use of the formula $\frac{\Delta}{1.87} \times 22.4$ = osmotic pressure in

atmospheres in which the symbol Δ refers to the depression of the freezing point of the solution in degrees Centigrade, below that of distilled water, 1.87 is the depression of the freezing point of a molecular solution of a nonelectrolyte, and 22.4 is the osmotic pressure in atmospheres of a molecular solution of a nonelectrolyte. There is given in Table 5 a calculation of the osmotic pressures in atmospheres of solutions showing depressions of the freezing points from 0 to −2.99°C.

In comparing various solutions as to their osmotic properties and effects, it is customary to speak of solutions having the same osmotic pressures as being isotonic. If a solution has an osmotic pressure that is less than normal or less than that of a solution with which it is being compared, it is called hypotonic. If the osmotic pressure of a solution is greater than normal or is greater than that of a solution with which it is being compared, it is called hypertonic.

TABLE 5.—OSMOTIC PRESSURE IN ATMOSPHERES FOR EACH HUNDREDTH OF A DEGREE CENTIGRADE DEPRESSION OF THE FREEZING POINT, FROM 0 TO −2.99°C.

	Hundredths of a degree centigrade									
−°C	0	1	2	3	4	5	6	7	8	9
0.0	0.000	0.121	0.241	0.362	0.482	0.603	0.724	0.844	0.965	1.085
0.1	1.206	1.327	1.447	1.568	1.688	1.809	1.930	2.050	2.171	2.291
0.2	2.412	2.532	2.652	2.722	2.893	3.014	3.134	3.255	3.375	3.496
0.3	3.616	3.737	3.857	3.978	4.098	4.219	4.330	4.459	4.580	4.700
0.4	4.821	4.941	5.062	5.182	5.302	5.423	5.543	5.664	5.784	5.904
0.5	6.025	6.145	6.266	6.386	6.506	6.627	6.747	6.807	6.988	7.108
0.6	7.229	7.349	7.469	7.590	7.710	7.830	7.951	8.071	8.191	8.312
0.7	8.432	8.552	8.672	8.793	8.913	9.033	9.154	9.274	9.394	9.514
0.8	9.635	9.755	9.875	9.995	10.12	10.24	10.36	10.48	10.60	10.72
0.9	10.84	10.96	11.08	11.20	11.32	11.44	11.56	11.68	11.80	11.92
1.0	12.04	12.16	12.28	12.40	12.52	12.64	12.76	12.88	13.00	13.12
1.1	13.24	13.36	13.48	13.60	13.72	13.84	13.96	14.08	14.20	14.32
1.2	14.44	14.56	14.68	14.80	14.92	15.04	15.16	15.28	15.40	15.52
1.3	15.64	15.76	15.88	16.00	16.12	16.24	16.36	16.48	16.60	16.72
1.4	16.84	16.96	17.08	17.20	17.32	17.44	17.56	17.68	17.80	17.92
1.5	18.04	18.16	18.28	18.40	18.52	18.64	18.76	18.88	19.00	19.12
1.6	19.24	19.36	19.48	19.60	19.72	19.84	19.96	20.08	20.20	20.32
1.7	20.44	20.56	20.68	20.80	20.92	21.04	21.16	21.28	21.40	21.52
1.8	21.64	21.76	21.88	22.00	22.12	22.24	22.36	22.48	22.60	22.72
1.9	22.84	22.96	23.08	23.20	23.32	23.44	23.56	23.68	23.80	23.92
2.0	24.04	24.16	24.28	24.40	24.52	24.63	24.75	24.87	24.99	25.11
2.1	25.23	25.35	25.47	25.59	25.71	25.83	25.95	26.07	26.19	26.31
2.2	26.43	26.55	26.67	26.79	26.91	27.03	27.15	27.27	27.39	27 51
2.3	27.63	27.75	27.87	27.99	28.11	28.23	28.34	28.46	28.58	28.70
2.4	28.82	28.94	29.06	29.18	29.30	29.42	29.54	29.66	29.78	29.90
2.5	30.02	30.14	30.26	30.38	30.50	30.62	30.74	30.86	30.98	31.09
2.6	31.21	31.33	31.45	31.57	31.69	31.81	31.93	32.05	32.17	32.29
2.7	32.41	32.53	32.65	32.77	32.89	33.00	33.13	33.25	33.36	33.48
2.8	33.60	33.72	33.84	33.96	34.08	34.20	34.31	34 43	34.56	34.68
2.9	34.79	34.91	35 04	35.16	35.27	35.39	35 51	35.63	35.75	35.87

Cryoscopy.—The usual method for determining the osmotic pressure of a solution is by cryoscopy—the determination of its freezing point as compared with that of distilled water. For this purpose a very sensitive thermometer is used, the scale of which extends only over about 5° and is divided into hundredths of a degree. The first step consists in an adjustment of the mercury in the thermometer so that when the thermometer bulb is immersed in freezing distilled water the mercury will stand somewhere near the upper limit of the 5° scale. Then place some distilled water in a test tube in which the bulb of the thermometer is inserted (see Fig. 7). This test tube is supported in a second tube with an air space between the two tubes on all sides. The larger tube is now suspended in the refrigerating vessel, which may be cooled by means of ice or by the rapid evaporation of ether. The liquid whose freezing point is being determined is constantly agitated by some stirring device so as to keep the whole mass of the liquid at the same temperature. The water as it cools will exhibit a temperature somewhat below the actual freezing point of the water, but as soon as ice forms in the liquid the mercury will rise in the tube, at first rapidly, and then more slowly, until it reaches a maximum. This maximum point marks the true freezing point of the water. Later the mercury may fall again. Careful note must be made of the exact reading of the thermometer while the mercury stands at the maximum point, for this indicates the true zero reading of the thermometer. The inner tube is now emptied of the distilled water and a sample of the liquid to be tested is put into the tube, care being taken to leave in the tube a few small crystals of ice to serve as centers of congealation. The process of freezing the liquid is now repeated. The difference between the two freezing points is the required depression of the freezing point and is usually designated by Δ. This method is the one most used for determining the molecular concentrations of organic liquids. It has some serious defects. It gives the values of the solutions examined at the freezing point, and not at the tem-

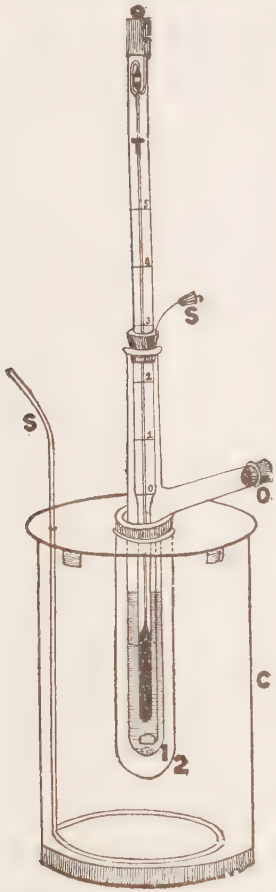


FIG. 7.—Apparatus for determination of the freezing point. *C*, container for freezing mixture of ice and salt; 1, test tube containing the substance to be tested; 2, large test tube to serve as a jacket; *O*, opening for introduction of liquid to be tested; *S*, *S*, stirring wires; *T*, delicate thermometer of which the fine graduations are not shown. (*From Mitchell, "General Physiology." By permission of the McGraw-Hill Book Company, Inc.*)

peratures at which body processes ordinarily take place. Organic liquids are easily altered and are exceedingly sensitive to minute differences of temperature. It is entirely possible that the information given by determination of the freezing point may sometimes be misleading on account of the abnormal condition involved in determining the physical constants of the solution.

Osmotic Pressure of Tissue.—As will be noted in another place, physiologists have given a great deal of attention to the osmotic pressure of body fluids, such as blood, lymph, urine, etc. Until recently, however, there had been few attempts to make an estimate of the osmotic pressures of different body tissues. Dr. Collip has given the measurements of the depression of the freezing points of several tissues of the dog (and cat), and has pointed out the possible significance of the figures obtained. Table 6, taken from his work, indicates the nature of the results.

TABLE 6

	In degrees Centigrade			
	Experiment 1, dog Δ	Experiment 2, dog Δ	Experiment 3, cat Δ	Experiment 4, dog Δ
Whole blood	0.61	0.64	0.63	0.82
Lymph	0.63			
Heart	0.77	0.81	0.86	
Lung	0.77	0.74	....	0.86
Spleen	0.77	0.75	....	0.87
Liver	..	....	....	0.91
Muscle (thigh abductor)	0.84	0.85	....	0.94
Brain	....	0.76		

It is to be noted that in each case the depression of the freezing point is greater for the tissue than for the blood or lymph. It is, of course, true that it is the liquid of the cell which must be in equilibrium with the liquid outside the cell. Measurements of the electrical conductivity of the cells made along with the cryoscopic determinations indicate that the conductivity of the tissue extracts is lower than that of the blood. In other words, while the osmotic pressure of the tissue is higher than that of the blood, it is due to a greater concentration of non-electrolytes in the cell than in the blood, while the concentration of electrolytes is greater in the blood than in the tissue.

The same author has shown that there is not only a condition of osmotic equilibrium existing between the blood and the tissue fluids, between the tissue and the cytoplasm of the cells, but there is also

an equilibrium between the cytoplasm and the nucleus in each nucleated cell. It must be understood that this statement does not of necessity imply that the osmotic pressure of the liquid on the two sides of the membrane shall be the same—simply that there is an equilibrium condition. Further amplification of this point will be found in a later place.

Physiologists have met two serious difficulties in calculating the molecular concentrations of solutions to be used in experimental work as substitutes for body fluids. Such solutions should, of course, be isotonic with the body fluids so as to avoid the purely mechanical effects of the solutions upon the tissues examined. One of these difficulties arises from the fact that many substances, such as sugar, for example, appear upon going into solution in water to form hydrates. When cane sugar is dissolved in water, a combination between solute and solvent molecules is evidently effected, each molecule of sugar combining with five or six molecules of water. Such combinations undoubtedly take place in the aqueous solutions of very many substances and serve to modify to a greater or lesser degree the diffusion pressure of the solvent. The degree to which such hydrations occur at the temperatures at which normal physiological processes go on has not yet been determined. The second difficulty arises from the ionization of electrolytes in solution. The degree to which such ionization takes place in solutions, of the molecular concentrations and composition of normal body fluids, has not been thoroughly investigated. In general, it may be supposed that the ionization constant for the salts of the body fluids is about 1.85.

Permeable and Semipermeable Membranes.—It will be recalled that a definition of a membrane has already been given, together with a suggestion as to some of the properties exhibited by membranes. There seem to be all possible gradations between membranes which will allow dissolved substances to pass freely through them and membranes of such a character as to hold back all dissolved substances and allow none to pass. The question as to why a membrane may be permeable to one substance and not to another is of great importance in physiology. It has a bearing not only upon the purely physical matter of dialysis and of osmotic pressure, but upon the equilibrium which exists between animal cells and their surroundings and upon the matter of absorptions of food and the getting rid of wastes.

Traube, the discoverer and student of various precipitation membranes, was of the opinion that the feature which enables a membrane to differentiate between one substance and another is the size of its intermolecular spaces. According to this suggestion, the membrane would then act in the capacity of a sieve and allow such substances to pass through as have molecules small enough to pass between the molecules of the substance composing the membrane. It is a fact that many of the substances held back by animal and plant membranes are such as

are known to have large molecules. There seem to be cases, however, in which this theory does not give a satisfactory answer to the question why some dissolved substance does not pass through the membrane. In order to account for the facts observed, the suggestion has been offered that, for a substance to pass through a membrane, it must be soluble in the substance of the membrane. This immediately brings up the question as to the nature of solid solutions, concerning which very little is known.

Factors Influencing Permeability.—Many experiments have been made and much speculation indulged in for the purpose of finding an explanation of the various phenomena associated with the transfer of dissolved substances from one side of the cell membrane to the other. Among the suggestions which appear to be worthy of serious consideration is that of Jacques Loeb, who holds that in the diffusion of electrolytes into the egg of Fundulus, so as to bring about an effect upon the heart, two conditions must be met. First, there must be an osmotic effect, *i.e.*, there must be an adequate concentration of the electrolyte (in this case potassium chloride) so as to provide the opportunity for a flow of the substance from a higher concentration on the one side of the membrane to a lower concentration on the other. In addition to the osmotic factor, there is, second, a salt effect. By this Loeb means that it is impossible for the potassium to diffuse through a membrane of the living Fundulus egg which had been previously freed from its salts by washing in distilled water for 24 hours, until the external surface of the membrane has again been supplied with salts, either by adding a second salt or by giving the dissolved potassium time to enter into combination with certain constituents of the membranes and thus supplying the general salt effect. The suggestion made is that the proteins of the membrane are dissociated by the salt (or acid), and it is through this ionization that diffusion phenomena are able to take place. This salt effect has been shown to play a rôle in the diffusion of substances through the membrane in both directions. The minimal quantity of a salt required to bring about the salt effect is a function of both the anion and the cation. In the anion the quantity required diminishes according to Hardy's valence rule, Chloride : Sulphate : Citrate = 1 : 4 : 16. In regard to the cation it was found that caesium and rubidium completely inhibit the diffusion of potassium chloride out of the eggs of Fundulus in M/2 solution. The salts of strontium and barium have similar effects. The salts of sodium, lithium, magnesium, calcium, ammonium, and $N(C_2H_5)_4Cl$ favor diffusion. Since the salts of lithium, ammonium, and $N(C_2H_5)_4Cl$ are extremely toxic for the embryo, the beneficial influence of these salts upon the recovery of the embryo from potassium poisoning must be ascribed to an action upon the membrane and not to an action upon the heart of an embryo.

The existence of a general salt effect can be demonstrated also for the diffusion of acid through the membrane of the egg of Fundulus. Very weak acid solutions can themselves supply the general salt effect, owing probably to the fact that acids form stable compounds with certain proteins of the membrane.

Loeb has also called attention to the fact that, when eggs are immersed in solutions of bases not sufficiently concentrated to injure the embryos, the bases become injurious when neutral salts in low concentrations are added to the solutions. Also, when Fundulus eggs are placed in acid solutions which kill the embryos in a short time the addition of a neutral salt has the effect of antagonizing the effect of the acid. The minimal quantity of a salt required for these salt effects diminishes with an increase in the valency of both anion and cation of the salt. It can be shown that the additive effect of salts to a base and the antagonizing effect of salt to acid are due in the case of eggs of Fundulus to an influence upon the rate of diffusion of alkali and acid through the membrane of the egg; this influence is accelerating for the diffusion of alkali and retarding for the diffusion of acid.

Direct Determination of Permeability.—There is not often an opportunity to examine a cell of sufficient size so that it is possible to make a chemical analysis of the content of a single cell. So far as the writer is aware there is no single animal cell which lends itself to this sort of experiment. The cells of one of the algæ, Valonia, are so large however, that it is possible to get from a single cell from 1 to 5 cubic centimeters of cell sap. This makes it possible to compare the chemical nature of the sap with that of the surrounding medium. It has been found, for example, that the metallic ion magnesium, so abundant in the sea water, is practically wanting in the cell sap. Potassium is found in the cell sap in much higher concentration than in the sea water. Sodium and calcium are equally demonstrable in both sea water and cell sap. With regard to the anions it may be said that chlorine is found abundantly both within and without the cell membrane. Sulphates, relatively abundant in the sea water, are practically absent from the cell sap. Nitrates also appear in the sap more than in the sea water. Thus, while nitrates and potassium are stored within the cell at higher concentration than in the surrounding sea water, magnesium and sulphates are excluded. The membrane in this case is to be considered as an osmotic membrane, but has no selective properties so far as permeability is concerned. The protoplasm within the membrane may also be selective in so far as choice of materials for the building up of new cell substance is concerned.

Applications of Dialysis and Osmosis in Physiology.—When it is considered that all the cells in the body are covered by membranes which are more or less semipermeable, it is clearly evident that all of the processes concerned in the nutrition of the body and in the getting rid of

wastes are directly dependent upon the laws governing permeability and osmotic interchange. The food materials upon which every living animal is dependent have to pass through the membranes of the cells of the alimentary tract into a body liquid of one sort or another, through other membranes into the cells which they are destined to nourish, etc. This is true of all the proteins, carbohydrates, and fats, the oxygen which is taken into the cells, the carbon dioxide and the various nitrogenous compounds which are given off from the cells, as the results of metabolism, and the internal secretions carried by the blood stream to all parts of the body. It seems to be essential that the fluids of the body should, in general, maintain a very constant concentration of salts and other substances which exert osmotic pressure, there being very little variation in this regard in any of the higher animals.

The question may be raised whether the absorption by the intestinal epithelium of substances in solution in the intestine, and their transfer to blood or lymph, can be explained wholly as due to filtration, diffusion, dialysis, or osmosis. It can readily be understood how solutions of low concentration may pass through the intestinal walls in accordance with the laws of osmosis as they are ordinarily understood. When, however, the osmotic pressure of the solution equals or is greater than that of the solution into which it diffuses there seems to be a contradiction. This apparent contradiction may, on the other hand, not prove to be a real one, for it has been shown that certain membranes exhibit a sort of one-sided permeability. This has sometimes been interpreted as permeability simply in one direction. Another suggestion appears to have in it more of probability—that the membrane is of such a sort that an equilibrium exists only when the solution on one side has a higher molecular concentration than that on the other side.

Similarly, the production of urine seems in many cases to contradict the most obvious physical chemical explanations. Lymph formation, too, seems to take place contrary to what have been believed to be the laws of osmosis and osmotic pressure. The difficulty here seems to the author simply another case of lack of knowledge of the ways in which cell membranes act. It has been assumed that they act just as some other membranes do. Cell membranes, however, unlike the membranes employed in many experiments upon osmosis, are colloidal in character. Not enough is yet known concerning the properties of colloids to predict just what a colloidal membrane may do under the varied conditions found in the animal body. When more has been learned about the properties of such membranes it may be found that all of these seemingly contradictory facts are readily explained.

CHAPTER IV

PROTOPLASM

Every study of physiological processes, from whatever point of view it may be made, leads of necessity to a consideration of protoplasm, the morphological substrate of living matter. The term protoplasm as originally used by Purkinje (1840) referred to the formative material of young animal embryos. In 1846 von Mohl used the term in the sense of the substance which forms the cell body as opposed to that of the nucleus, or what is now called cytoplasm. Following the lead of Strassburger (1882), biologists now consider protoplasm to be the essential substance of both nucleus and cell body of both animals and plants. It is now regarded as the only form of matter in which or by which the phenomena of life are manifested.

Protoplasm exists in many modifications. Ordinarily it is a thick, viscous, semifluid, and almost jelly-like, colorless, translucent material containing a large percentage of water, and holding granules in suspension. It coagulates when heated to about 60°C., and also under the influence of alcohol, strong acids, alkalies, and some salts. The chemical composition of the substance is not definitely known, but dead protoplasm has been shown to be composed largely of proteins. The living substance is probably very variable in its composition, and it is not likely that there is such a thing as a definite protoplasmic molecule, though the suggestion has more than once been made.

Structure of Protoplasm.—Investigators of the structure of living matter have formulated a variety of theories as to the architecture of the substance. It seems necessary to call attention to only two of these, since ideas concerning the structure of protoplasm have been somewhat clarified through the investigations of the past few years.

1. *The Reticular Theory.*—This theory supposes that the living substance is made up of a more fluid portion which fills in the meshes of a network comprising a more solid portion. The evidence in support of this view has been derived chiefly from a study of protoplasm which has been acted upon by powerful fixing agents. Such material, colored by various staining agents, presents appearances which would seem to indicate the presence of a definite fibrous network. It must be recognized that such material is not in its natural condition and that it is certainly possible that the material treated in this way may present appearances entirely foreign to what exists in the same material while in its living

condition. Hardy has made an intensive study of the structure of protoplasm as influenced by various reagents, and as the result of his work made the following summary statement:

> Cell protoplasm reacts to corrosive sublimate and osmic vapor in the same way as does a soluble colloid to a reagent which converts it into an insoluble colloid. No evidence that the structure discoverable in the substances of these cells after fixation has any counter part in the cell while living is found. The nucleus and attraction sphere represent something which is present in the living cell, but it is difficult to decide what may be due to the action of fixation.

The Alveolar Theory of Protoplasmic Structure.—It has fallen to the lot of two of our American investigators to give the best accounts of the structure of protoplasm. The method of study employed, that of the actual dissection of the protoplasm under the highest powers of the microscope, was originated by Dr. Kite and has been modified and improved by Dr. Chambers and others. Naturally, a considerable part of the earlier work of these investigators was given to an estimation of the possibilities and limitations of the method. There are many optical difficulties in the way of a clear understanding of the structure of the substance under investigation, due to reflection, refraction, diffraction, absorption, dispersion-interference, and the scattering action of light. Also it has been found that the optical image of the cell is a diffraction pattern, produced by the cell, and which may be, under certain conditions, very different from the object itself.

The dissections of the protoplasm are made in moist chambers, open at one side for the introduction of the dissecting needles employed in the operation. The cell to be studied is mounted in a small drop of liquid on the under side of the cover slip which covers the moist chamber. This permits the cells to carry on perfectly normal respiratory activities during the whole course of the study.

As a result of the investigations of these men it may now be said that living protoplasm has the nature of a hydrophyllic emulsion colloid. Like other emulsions, it may vary within very wide limits as to its water content. Like other emulsions, it sometimes appears in the more fluid sol condition and sometimes in the less fluid gel condition. Only as the nature of colloid substances and their behavior under definitely known and controlled (though possibly changing) physical and chemical conditions have become known has it been at all possible to explain in any satisfactory manner the varied appearances assumed by living matter.

Chemically, protoplasm contains a variety of proteins, possibly other organic colloidal substances, and inorganic salts in solution in water. Or perhaps it might be more nearly the truth to say that the emulsion which is called living matter consists, as do other emulsions, of two phases, the continuous phase and the dispersed phase. It has been cus-

tomary to speak of emulsions of oil in water, in which the small particles of oil are dispersed throughout the extent of the continuous phase. It has also been found possible to make emulsions in which the phase relations between the oil and water are reversed, so that minute droplets of water are dispersed throughout the extent of the continuous phase of oil. In protoplasm it seems likely that an emulsion is being dealt with in which the phase relations are still more complicated. Here it may be assumed that the continuous phase is a thin solution of proteins and inorganic salts in water. The particles of the dispersed phase may be assumed to be very thick, jelly-like solutions of water in proteins. It is probably true also that there is a constant shifting of the water balance in the protoplasm so that there is a transfer of water from the continuous phase to the particles of the dispersed phase and *vice versa*.

The facts upon which such an understanding of the structure of protoplasm is based are essentially as follows:

Dr. Kite found as the result of numerous dissections of the protoplasm of Amœba that:

> Small pieces of the ectoplasm of Amœba can be cut off in distilled water and show no change. This living substance has moderately high viscosity and cohesiveness; it does not stick to glass needles very readily, and very little difficulty is experienced in cutting it into pieces as small as the limit of microscopical visibility. Pieces of all sizes appear to be perfectly homogeneous. The cloudiness of the ectoplasm is a well-known property. The three or four inner microns of the hyaline ectoplasm, and particularly the interior of the outer ends of pseudopodia, contain numbers of small granules and globules that may measure as much as 3 or 4 microns. If these granules are dissected out they do not go into solution. The globules show confusing diffraction rings but both granules and globules can be brought out by light staining with diethyl-safranin-azo-dimethylanaline. The endoplasm contains a large contractile vacuole in which the presence of protein has not been demonstrated, as yet, and numerous food vacuoles which contain either liquid or food and liquid contents. The same kind of granules and globules are found in the endoplasm as are found in the ectoplasm and the number of these structures varies in different animals. The substance forming the walls of the vacuoles is of much higher viscosity and cohesiveness. The living endoplasmic substance is a very dilute and apparently homogeneous gel that possesses a remarkable affinity for water. The ectoplasm of the Amœba is, then, a quite concentrated gel, while the interior is quite dilute and is constantly changing its water-holding power in different regions. New methylene blue R and trypan blue are of great value in bringing out the globules, granules, and vacuoles. The nuclear membrane is an extremely thin and moderately tough solid substance. It shows some elasticity and is quite viscous.
>
> The whole of the nuclear substance is a highly rigid and granular gel, the minutest pieces of which show no appreciable change when dissected out in distilled water. A slight elasticity and a definite glutinicity are exhibited by this substance. There are variations in the concentrations of the nuclear gel that produce a characteristic but misleading optical image. The nucleus appears

to contain an irregular network with granules embedded within it. The interstices of the network are very small luminous spots which have been interpreted wrongly to be vacuoles. Many dissections have shown that the granules are very concentrated masses of gel, the network irregularly disposed masses of a diluter gel, and the interstices and light spots the most dilute gel in the nucleus. The so-called network is part of the nuclear gel that forms part of a concentration gradient; the interstices and granules are constants connected by the grading network. It should be clearly understood that the network is not made up of definite threads and fibers but of irregular masses of hydrogel that are very dense immediately surrounding the granules, from which they grade into the dilute gel of the interstices. No free liquid is found in the nucleus. When the granules are in focus they appear gray or cloudy or opalescent. When they are out of focus they appear as dark spots. They measure from less than 1 to about 2 microns in diameter.

It seems that a part of the luminosity of the interstices of the network is due to diffraction and not simply to a slight absorption of the light by this portion of the nuclear substance. The structural details of the nuclear substance can be brought out with considerable vividness by staining with Janus green.

Slight cuts upon the surface of the Amœba quickly close. Extensive cuts cause Amœba to explode. If the contractile vacuole be cut and its liquid contents be caused to mix with the protoplasm, the Amœba is immediately destroyed with explosive violence. In as short a time as 2 seconds nothing but the nucleus may remain. A relatively large dose of distilled water and even one-half to one molar cane sugar solution or one molar of sodium chloride or potassium nitrate gives similar results. Tests made with a large number of indicators show that the protoplasm is slightly alkaline in reaction.

Protoplasm a Hydrophyllic Emulsion Colloid.—According to Chambers, protoplasm is considered a hydrophyllic emulsion colloid consisting of an aggregate of albuminous granules dispersed through an aqueous medium in which electrolytes and non-electrolytes are held in solution. In early germ cells, egg cells, and protozoa the protoplasm usually exists in the sol state with a surface layer in the gel state. Adult somatic cells appear to be gels in which it is not easy to distinguish a cell membrane having a consistency different from that of the cytoplasm within. In general, the substance is homogeneous to the eye and may be liquid or solid according to the greater or lesser amount of the contained water.

The cytoplasm is filled with granules of at least two sizes and droplets of non-miscible fluids. The granules appear to be of two sorts: (1) microsomes, less than 1 micron in diameter, which constitute the most resistant parts of the cell. These particles are capable of maintaining themselves after complete disorganization of the cell. (2) Macrosomes, 2 to 4 microns in diameter and very sensitive to injury. These larger granules often coalesce or are drawn out into threadlets which may be crooked or straight, curled or lumpy. Generally speaking, none of these structures is persistent; they disappear and reappear and can be interpreted as localized changes in the physical state of the colloids composing

the protoplasm. Chambers studied numerous types of cells and found extreme variability in the consistency of the protoplasm due, in part at least, to the variation of the water content of the cell protoplasm.

The external surface layer of the cell is in the case of many cells a gel decidedly more rigid than the interior of the cells. This surface gel is very extensile and contractile and regenerates easily on injury. Sudden tears of the surface result in a pouring out of the internal protoplasm and dissolution. In some cases at least the external gel grades off rather insensibly into the sol in the interior of the cell.

The nucleus of the cell is a surprisingly more rigid gelatinous substance than the cytoplasm in which it lies. Between the nucleus and the cytoplasm is a thin, structureless, nuclear membrane. Chambers was also able to see in the germ cells of insects nuclei which, when injured, show a gradually increasing condensation or separation of granules which collect from an extensive network throughout the nucleus. These granules arrange themselves into a constant number of filaments which shorten and go through all the changes in shape and form that have been described in the examination of the chromosomes of fixed material. These artificially induced chromosomes as well as the chromosomes of a normally dividing cell are a highly viscous and glutinous gel.

Disorganization of the egg cells studied may take place as follows: The macrosomes swell and go into solution; the liquid hyaline cytoplasm may flow out and disappear in the surrounding water or it may set, forming a rigid coagulated mass. The coagulation structure gradually coarsens and produces a network or granular precipitate. Injury is accompanied by swelling and an apparent increase in the acid reaction of the part involved. Certain observations made by Chambers upon cells undergoing division are of interest. The interior cytoplasm of a marine egg is a fluid whose viscosity is high enough to prevent Brownian movement of the enclosed granules but low enough to allow their ready dislocation by means of the needle. The formation of the aster consists in the gelation of the hyaloplasm under the influence of the astral center. A hyaline liquid separates during gelation and flows in innumerable pathways toward the center, where it accumulates to form the sphere. This centripetal flow arranges the gelling hyaloplasm containing the cell granules into radial strands. The strands merge peripherally into the surrounding liquid cytoplasm, or reach and anchor themselves in the substance of the gelled superficial layer of the egg when the aster is fully formed.

During cell division a periodic reversal of the sol and gel states occurs in the following order: (1) the cytoplasm is a gel when the aster is fully formed; (2) it reverses to a sol as the radiations fade out and the sphere liquid collects at the two poles of the nucleus; (3) the appearance of the amphiaster heralds a return to the gel state; (4) at the equator of

the cell the cytoplasm reverses to the sol stage as a constriction develops cutting the cell into two.

Reversals of Phase.—The gel state in living protoplasm is not inert even to the eye. A reversal of the gel to the sol state is easily brought about by tearing. In discussing the structure of protoplasm from the colloid standpoint it is necessary to call attention to an analogy in the cases of emulsion of oil and water. Usually, an emulsion of oil in water is referred to, but under certain conditions it is possible to maintain emulsions of water in oil. For the present purpose, it does not seem necessary to present all of the facts of the case, interesting though they are In protoplasm a substance is found, in the nature of an emulsion, which presents two phases, a more solid and a more liquid. The first of these

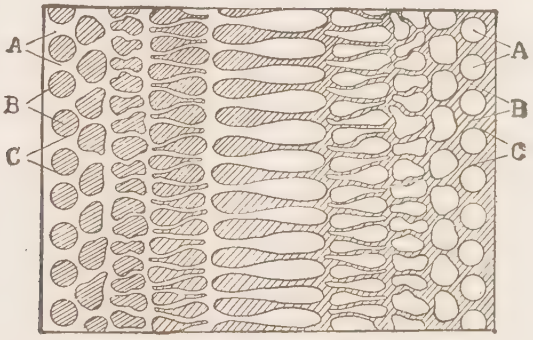


FIG. 8.—Diagram of a colloidal emulsion illustrating the transformation of an emulsion of oil in water to an emulsion of water in oil. *A*, aqueous phase; *B*, oil or other non-aqueous phase; *C*, space film of soap or other dispersing agent. (*After Clowes*, 1916. *From Sharp, "Introduction to Cytology." By permission of the McGraw-Hill Book Company, Inc.*)

phases appears to be a sol of water in protein and the second a sol of protein in water. It is clear how the different appearances which Chambers has described may be explained. The more solid parts of the protoplasm maintain their integrity for a time and then gradually go through a change of phase, from that of water in protein to protein in water. The reversed process also takes place in other parts of the cell.

These changes of phase are apparently entirely comparable to the changes of phase taking place in emulsions of oil and water under certain conditions. Ordinarily, an emulsion of oil in water consists of very minute droplets of oil dispersed through the water phase. By the addition of some dispersing agent to the emulsion it is possible to invert the phase so that the emulsion will become one of water in oil. Figure 8 is a diagram representing the transformation of an emulsion of oil in water to an emulsion of water in oil. It is entirely possible that the change of phase in case of proteins is not as simple as that which takes place in the oil-water emulsion. What the determining cause for these

changes is has not yet been discovered with certainty. Evidently, the chemical processes (metabolic) taking place in the cell have something to do with these changes. The chemical constitution of the cell is constantly undergoing change, and as these changes take place there are corresponding changes in the structural phases of the substance.

The following statement from a paper by Heilbrunn is significant of our present understanding of the structure of protoplasm:

> As a colloidal system, protoplasm is exceedingly complex . . . Chemically, protoplasm is known to consist of a variety of substances: proteins, fats and lipoids, carbohydrates, and inorganic salts. Cytological study has helped to locate these substances within the cell. It is generally admitted that protoplasm is a watery solution which contains dissolved in it salts, and perhaps carbohydrates. The proteins are in a state of colloidal solution, but not completely so, for many of the granules found in protoplasm are doubtless protein and these are in suspension. The fats and lipoids are usually emulsified and are scattered through the cell as tiny droplets which apparently constitute most of the mitochondria. The protoplasm is at one and the same time a true solution, a colloidal solution, a suspension, and an emulsion.

The small masses of protoplasm called cells behave in one respect as do other drops of liquid material They cohere to form a system of drops which do not fuse with each other only when they are separated from each other by thin films of material which is insoluble in either the substance of the drops or of the surrounding fluid. A very interesting example of this is the emulsion structure formed when a few drops of 10 per cent solution of lecithin in chloroform are shaken together with 10 per cent solution of egg albumen in water in a test tube. When these substances have been shaken together so as to form an emulsion and some of it has been poured out upon a slide or a watch crystal and allowed to evaporate, it is found that the rather finely divided emulsion remains unchanged for some time. There has been formed between the chloroform and albumen phases a wall which dissolves only with difficulty.

Chemical Composition of Living Matter.—As has already been intimated, the elementary substances entering into the composition of protoplasm are the same elements which form a very large part of inorganic matter. All the substances entering into the composition of this living matter are those known to the inorganic chemist. Of the eighty-seven elements, thirty-five have been found to enter into living matter. Seventeen appear to be essential to life and not more than four constitute from 96 to 99 per cent of the living organism. These elements, which comprise the living substance, are sometimes referred to as bioelements. Table 7 gives the percentage composition of the living matter of a mammal (man), the percentage composition of a gymnosperm (pine), and the average percentage composition of the three great classes of foodstuffs. A glance at Table 8, copied from Millikan's "The Electron," will show

that the most abundantly represented elements are those having the lower atomic weights. The exact significance of this fact is a matter of speculation.

Of all the elements composing living matter, oxygen is the only one which enters in its elementary form. All the others are taken in and appear as compounds, usually in the case of animals, of very complex form. It is probably true that it is not correct to speak of protoplasm as a definite chemical substance in the same sense as the substances of inorganic chemistry are spoken of as definite substances. Protoplasm is rather a mixture, a sort of supercompound, of an indeterminate and variable composition. Among its chief components are the proteins, compounds of carbon, oxygen, hydrogen, and nitrogen. They are invariably substances of high molecular weight. The exact composition of the proteins is not known in any considerable number of cases. It is probable that the proteins as well as the combinations of proteins, salts, etc., forming protoplasm, are, while closely similar, specific for different species of animals. That is, the fundamental difference between one

TABLE 7.—DISTRIBUTION OF THE BIOELEMENTS

	Mammals	Gymnosperms	Carbohydrates	Average composition of foods		
				Fats	Proteins	Fresh water
1	O 62.43	C 63.96	O 49.38	C 69.05	C 51.3	O 88.80
2	C 21.15	O 38.65	C 44.44	O 17.90	O 22.4	H 11.16
3	H 9.86	H 7.13	H 6.18	H 10.00	N 17.8	S 0.018
4	N 3.10	Al 0.065	.....	P 2.13	H 6.9	C 0.005
5	Ca 1.90	Si 0.057	.....	N 0.61	S 0.8	Ca 0.004
6	P 0.95	S 0.52	.....	S 0.31	P 0.7	Mg 0.003
7	K 0.23	Fe 0.30	.....	.....	Fe 0.1	N 0.002
8	S 0.16	N 0.03	.....	.....	.....	Fe 0.002
9	Cl 0.08	Ca 0.007	.....	.....	.....	P 0.001
10	Na 0.80	K 0.006	.....	.....	.....	Na 0.001
11	Mg 0.027	P 0.005	.....	.....	.....	K 0.001
12	I 0.014	Mg 0.003	.....	.....	.....	Remainder
13	Fe 0.009	Cl 0.002	.....	.....	.....	0.003
14	Fe 0.005	Na 0.001				
15	Br 0.002	Fe 0.001				
16	Al 0.001	Mn 0.001				
17	Si 0.001					
18	Mn 0.001					
	100.000	100.000	100.00	100.00	100.00	100.00

TABLE 8.—THE ELEMENTS, THEIR ATOMIC NUMBERS, ATOMIC WEIGHTS, AND CHEMICAL POSITIONS (*Millikan* Modified). (*By permission of the University of Chicago Press*)

1 H 1.008											
2 He 3.99	3 Li 6.94	4 Be 9.1	5 B 11.0	6 C 12.00	7 N 14.01	8 O 16.00	9 F 19.00				
10 Ne 20.2	11 Na 23.00	12 Mg 24.32	13 Al 27.1	14 Si 28.3	15 P 31.04	16 S 32.06	17 Cl 35.46				
18 A 39.88	19 K 39.10	20 Ca 40.07	21 Sc 44.1	22 Ti 48.1	23 V 51.0	24 Cr 52.0	25 Mn 54.93	26 Fe 55.84	27 Co 58.97	28 Ni 58.68	
	29 Cu 63.57	30 Zn 65.37	31 Ga 69.9	32 Ge 72.5	33 As 74.96	34 Se 79.2	35 Br 79.92				
36 Kr 82.92	37 Rb 85.45	38 Sr 87.63	39 Y 88.7	40 Zr 90.6	41 Nb 93.5	42 Mo 96.0	43—	44 Ru 101.7	45 Rh 102.9	46 Pd 106.7	
	47 Ag 107.88	48 Cd 112.40	49 In 114.8	50 Sn 118.7	51 Sb 120.2	52 Te 127.5	53 J 126.9				
54 X 130.2	55 Cs 132.81	56 Ba 137.37	57 La 139.0	58 Ce 140.25	59 Pr 140.6	60 Nd 144.3	61-62 Sm 150.4	63 Eu 152.0	64 Gd 157.3	65 Tb 159.2	66 Ds 162.5
67 Ho 163.5	68 167.7	69 Tu 168.5	70 Yb 173.5	71 Lu 175	72-73 Ta —181.5	74 W 184.0	75—	76 Os 190.9	77 Tr 193.1	78 Pt 195.2	
	79 Au 197.2	80 Hg 200.6	81 Tl 204.0	82 Pb 207.2	83 Bi 208.0	84 Po 210.0	85—				
86 Em 222.0	87—	88 Ra 226.0	89 Ac (227)	90 Th 232.15	UrX 2 234	92 Ur 238.2					

In this table of the elements those which seem to be of greatest abundance and importance in living matter are underlined ═══; those somewhat less abundant, but of great importance ═══; those occurring in still smaller amounts, or only occasionally - - -

species of animal and another lies in the fact of slight though definite differences in the composition of the (protein) substances and the plan of architecture according to which these substances are arranged.

The immensely complex character of the protein constituents of protoplasm may be indicated by a single example. Professor Latham has shown the probable composition of albumin to be

C	H	N	O	S
240	517	65	75	3

Those interested may refer to his papers in vol. 3 of the *Biochemical Journal* for evidence as to the primary substances involved in building up this great molecule. This molecule with a weight of 800 is small as compared with many others, as is indicated by freezing-point determinations from which estimates of their molecular weights may be made.

The Biogen Hypothesis.—In connection with the discussion of the chemical composition of protoplasm, there should be mentioned the biogen theory of Verworn, on account of the place it has held in biological literature. Verworn supposes living matter to be made up of very large and relatively unstable molecules, vastly more complex than the protein molecules. These are considered to have a central permanent nucleus and a great number of side chains. These side chains are capable of oxidation, reduction, etc. The addition of oxygen is conceived to bring the molecules to the maximum of their instability, or power of decomposition. This follows the taking from the food matters of certain atomic groups from the proteins and their placement in the biogen so as to form certain cyanogen-like compounds. After the addition of the oxygen, the molecules, partly spontaneously and partly as the result of stimulation, break down more or less explosively, causing the formation of carbon dioxide and other waste products. These large molecules, biogen molecules, are considered the physical basis of the phenomena called life. The changes which take place in the breaking down of these compounds and their reconstitution are what is ordinarily called the vital phenomena.

Verworn's hypothesis has not met with general acceptance. The great variety of forms in which living matter appears seems to lend weight to the idea that the living substance is rather a mixture of molecules than anything so definite as a chemical compound. It may well be asked whether such a substance as protoplasm actually exists as a chemical entity. To quote from Sir Michael Foster:

> Does it wax and wane as does a crowd, the units constituting the whole inconstant in number but identical in nature, or is it a material unstable alike in form and amount? He (the biologist) may speak of protoplasm as a complex substance, but he must strive to realize that what he means by that is a complex whirl, an intricate dance of what he calls chemical composition, histological structure, and gross configuration are all, so to speak, the figures; to him the renewal of the protoplasm is but the continuance of the dance, its functions and actions the transference of figures.

Protoplasm an Heterogeneous System.—It has already been pointed out that protoplasm is an extremely heterogeneous system. It is made up of numerous components, some of which are crystalloidal, and some of which are colloidal in character. These substances may be very unlike, and they may be such as readily and strongly interact if they are brought together under the influence of some sort of catalytic agent. It has been mentioned elsewhere in this work that the origin of living matter was probably associated with the existence of the colloidal condition. That is, the existence of heterogeneous systems of the general character of

some of the inorganic colloids made possible those chemical changes which later gave rise to self-perpetuating systems. The colloidal system is a system in which energy exchanges may readily take place; it is a system of great instability and great irritability. A very small variation in condition may induce great energy transformations. This is especially true in enzyme or other catalytic actions.

CHAPTER V

PROPERTIES OF PROTOPLASM

Protoplasm exhibits a few characteristics by which it may always be recognized and distinguished from other material substances. These are its chemical composition, metabolism, its tendency to undergo cyclic changes, and its power to produce its own catalytic agents.

1. *Chemical Composition.*—Protoplasm always contains one or more forms of a complex compound of carbon, oxygen, hydrogen, nitrogen, the so-called protein, a substance which has never been obtained except as the product of living things, and which, united with a large proportion of water, forms a large part of the living substance.

2. *Metabolism.*—The living substance is constantly undergoing change. There is catabolism, a universal waste and disintegration of the protoplasmic substance through oxidation, and at the same time anabolism, a reintegration by the intussusception of new matter. Molecules are being constantly oxidized and then cease to be parts of the living body. Carbon dioxide is one of the substances given off, while others contain the other elements entering into the composition of protoplasm. If the two processes, anabolic and catabolic, are equivalent in amount, an equilibrium of mass results. If more body material is built up than is dissipated, there is an increase in mass. If more body material is broken down than is built up in a given space of time, there is a loss of mass.

3. *Tendency of the Protoplasm to Undergo Cyclic Changes.*—All living matter procedes from preexisting living matter, a portion of the latter being detached. This acquires an independent existence. Food matter, in either the inorganic or the organic form, is taken in and provides, among other things, the material out of which new living substance is built. The new organism takes on the form and exhibits the characters of that from which it arose. It, within very narrow limits of variation, leads the same sort of life and does the same sort of work as other members of the same group. It exhibits the same power to propagate itself by means of an offshoot. Sooner or later its life processes cease and, like its predecessors, it is resolved into more highly oxidative compounds of its elements. Its parts are now in position again to enter the life cycle, and may do so in any one of a great multitude of plant and then of animal forms.

4. *Power to Produce Its Own Catalytic Agents.*—The chemical operations of the cells are carried on by means of enzymes, each of which appears in control of a given reaction. The power to produce the great variety of enzymes necessary to do the work of any organism, as well as the many kinds of enzymes which appear to serve in making different protoplasms specific as to kind, would appear sufficiently fundamental to be termed diagnostic.

The Fundamental Properties of Living Matter.—All living matter exhibits certain characters which, because they are common to all cells alike, are spoken of as the fundamental properties of living matter. Some of these will overlap some of the characters which have already been mentioned, as being diagnostic of living matter. It may be said that certain of these properties appearing alone would not be sufficient to determine a given structure as living rather than dead, but it may be held that if all or any considerable number of them can be demonstrated, the material would be classed as living matter.

These properties are:

Irritability.
Conductivity.
Contractility.
Spontaneity (?).
Metabolism.
Rhythmicity.
Maintenance of high temperature (?).
Growth.
Consciousness.
Memory.
Reproduction.

Irritability.—A very casual examination reveals the fact that many cells and tissues are sensitive to changes in their immediate environment. Closer study reveals the fact that such a quality is characteristic of all living cells. The nature of the response by which it is learned that some environmental change or condition has made an impress upon a cell will vary according to the type of the individual cell, as well as the particular conditions under which the stimulation takes place. The kind and degree of differentiation which the cell has undergone during the course of its development will give a clue to the nature of the response which will be seen. For example, the muscle cell will respond to a stimulus by a contraction in a definite direction. The gland cell will respond to stimulation by producing a secretion. The nerve cell will give origin to a nervous impulse. The egg cell will respond to certain forms of stimulation by undergoing cell division.

Irritability involves some fundamental change in the rate or kind of processes going on within the cell or through the cell membrane. These changes may be either chemical or physical—or may be both. The protoplasm of the cell must be considered as being in a constant condition of change. The very fact that the cell is alive means that certain changes are taking place. It is possible to believe that in a resting cell the various processes of the cell take place at rates which are adjusted to each other

in a very definite way. Any disturbance of the cell has the effect of altering in some degree some of the processes going on within the cell, and probably alters different processes in different degrees, so that as the result of the change in outside conditions there is a serious disturbance of the balance or the equilibrium of the cell. Any such disturbance of equilibrium of the cell leads immediately to a change in the cell's behavior, and this change in behavior is called a *response*. Every alteration in the external conditions of the cell may bring about such a change in behavior as has been mentioned and may, therefore, be called a *stimulus*.

Classification of Stimuli.—Stimuli may be classified in various ways. A great variety of external conditions may alter the behavior of a cell and these different sorts of stimuli may differ among themselves in quality, in direction, in amount, in duration, and in frequency. Accordingly, stimuli may be classified as follows:

1. Quality:
   - Chemical
   - Thermal
   - Photic
   - Mechanical
   - Osmotic
   - Electrical
2. Direction:
   - Positive
   - Negative
3. Amount:
   - Threshold of stimulation
4. Duration:
   - Length of time of application
5. Frequency:
   - Rapidity with which stimuli are applied

Nature of Irritability.—A number of theories have been suggested as to the nature of irritability. None of these has proved to be acceptable to all physiologists, so the essentials of some of the theories which today appear most likely to lead toward the truth may be simply stated briefly.

In 1899, Dr. Jacques Loeb outlined a theory of irritability which may be summarized as follows: The salts, or electrolytes in general, do not exist in the tissues as such exclusively, but are partly in combination with proteins (or fatty acids). The salts or electrolytes do not enter into combinations as a whole but through their ions. The great importance of these ionprotein compounds (or soaps) lies in the fact that by the substitution of one ion for another the physical properties of the physical compounds change, for example, their surface tension, their power to absorb water, or their viscosity, or their state of matter. There are thus

in these ionprotein compounds or soap compounds essential constituents of living matter, which can be modified at desire, and hence it is possible to vary and control the life phenomena themselves.

Life phenomena, and especially irritability, depend upon the presence in the tissues of a number of various metal proteins or soaps (Na, Ca, K, and Mg) in definite proportions. Quoting again from Dr. Loeb:

If we finally sum up the results of these observations, we come to the conclusion that all those phenomena which depend upon the action of muscles, nerves, or glands seem to be influenced to a large extent by the salts, and that especially changes in the proportion of Na and K to the Ca ions in the tissues seem to affect their properties and their actions. The idea to which we have given preference, namely, the substitution of Na or K for Ca or *vice versa*, in certain organic compounds, gives rise to a contraction, may possibly have to be modified in detail, and undoubtedly many new facts will be required and found before we are ready for a final theory, but I am inclined to believe that the main structure will remain as intimated in my papers of 1889 and 1900, namely, that the normal qualities, especially the normal irritability, of animal tissues depend upon the presence in these tissues of Na, K, Ca, and Mg ions in the right proportion; that these ions are at least partly in combination with colloids (proteins or higher fatty acids or possibly carbohydrates), and that any sudden change in the proportions of these ion lipoids, ion proteins, or ion carbohydrates alters the properties of the tissues and gives rise to an activity or an inhibition of an activity according to the sense in which the change takes place. Finally I believe that the natural rhythmic processes, such as heart beat, respiration, etc., are due to the substitution of certain metal ions for others, these substitutions being caused by enzymotic processes going on continually, and by which, among others, metal ions are freed from certain combinations, and rendered available for others, as seems to be the case in the action of rennet in the coagulation of milk. We certainly understand by this hypothesis why the combination of Na-, K-, Ca-, and possibly Mg- salts is so important for the life of the animal.

Lillie's Theory of Irritability. — Dr. Ralph S. Lillie has presented a theory of irritability based upon changes in the plasma membrane of the cell following stimulation. It is conceivable that the plasma membrane may play the part simply of a protecting or insulating membrane while the distinctly irritable mechanism lies within the cell in the living protoplasm. Dr. Lillie holds that a stimulus, in order to act upon a cell, must at first affect the surface of the cell. He maintains that the membrane is the essentially irritable part of the cell, playing the rôle of a sensitive intermediary between the external world and the internal protoplasm, so that alterations in the membrane, particularly of its permeability, necessarily involve changes in the metabolism and hence in the energy production of the cell.

Any increase in the ionic permeability of the membrane produces a corresponding change in its polarization, that is, a depolarization which may evidently vary in degree. This depolarization is supposedly an

essential part of the process, and there are reasons for supposing that it and not the mere increase in permeability as such is the really critical change in stimulation.

In speaking of chemical stimulation as a special case of stimulation Lillie says that the primary change in the stimulation of an irritable tissue is a sudden increase in the permeability of the boundary layers or plasma membranes of the constituent cells or elements. The resistance to escape of diffusible substances, including carbon dioxide, is thus diminished and there results a corresponding acceleration of energy, yielding oxidation. Chemical stimulation results from those substances which affect the constituents of the plasma membrane in such a manner as to increase its permeability to the degree required. The plasma membrane is primarily a colloid structure consisting of proteins and lipoids intimately mixed, possibly intercombined. It would therefore be expected that its structure or consistency would be altered and its permeability increased or decreased by substances which influence colloidal aggregation states.

Chemical stimulation may be either reversible or irreversible depending upon the reversibility or irreversibility of the changes produced in the plasma membrane. The membrane may lose its vital semipermeability temporarily in the first case and permanently in the second. Alkali and alkali-earth neutral salts produce reversible changes in the colloidal aggregation state, while with heavy-metal neutral salts the aggregation changes are irreversible.

Conductivity.—The property of protoplasm known as conductivity is one through the action of which an impulse applied to one portion of a cell is transmitted through or over the whole of the protoplasm in such a way as to bring about the reaction of distant parts of the cell. For example, a stimulus applied to the surface of such a cell as Vorticella is conducted through the protoplasm, and the fact of such conduction is evidenced by the contraction of the long stalk. Or a stimulus may be transmitted through the protoplasm of the cell and result in the formation of a secretion, the sending out of a nervous impulse, etc.

There has been and still is a question whether conductivity is to be considered a property separate and distinct from contractility. The bulk of opinion seems to be in favor of considering it a distinct property.

Contractility.—All living matter exhibits to a greater or lesser degree the power of movement, so as to bring about a change of form in itself, or to move itself from one place to another. The term has been used chiefly to indicate changes in the forms of such structures as muscle cells, and it has sometimes been overlooked that these changes may be very slight indeed.

At least three different manifestations of contractility may be mentioned: amœboid movement, ciliary action, muscular action. The

movements of flagella and of undulatory membranes perhaps form special cases of one of the above.

Contractility is evidently due to the play of purely physical forces. Surface-tension changes and the imbibition of water by colloids, itself possibly a surface-tension phenomenon, have been invoked to explain the facts observed. McClendom attempts to explain amœboid movement as due to local increase in permeability of the plasma membrane. He calls attention to the fact that all those agents which stimulate muscle or nerve also cause a rounding up of an Amœba. This approach to the spherical form may be interpreted as due to an increase in the tension of the plasma membrane, following decrease in polarization, which is the result of increase of permeability to certain ions or classes of ions. It would be expected therefore that most of the tropic responses of the Amœba would be negative—as, indeed, they are. The Amœba avoids the region from which a strong stimulus comes because the permeability and the surface tension of the plasma membrane on that side are increased and the protoplasm flows in the opposite direction.

Spontaneity.—As has already been suggested, one of the great battle-fields of modern physiology has been the question whether the living organism may assume automatic changes in structure or in function apart from the influence of external conditions or stimuli, that is, whether it is simply a machine which acts only in response to stimuli or forces of one sort or another which are brought to bear upon it from without.

Only the most careful and painstaking work will ever be able to give a satisfactory and convincing answer to the question. One might be led to believe that some of the simplest organisms are controlled by impulses coming to them from without, that they are forced to do the things they do, that they are not able to yield to their likes and dislikes, if it is granted that such sensations exist at all. But it is not so easy to believe that the higher animals are not in some measure at least able to act in a way not to be accounted for by the reactions to purely physical external forces. It is difficult, for example, to believe that all the acts or thoughts of man are to be accounted for upon the basis of reactions to stimulation. The very fact that it is difficult for us to put ourselves in the frame of mind necessary to acknowledge the possibility of such a condition makes it all the more necessary that we should examine all the evidence in the most judicial spirit, and that we should be willing to accept as facts all that can bring a sufficient proof. There can be no question that the work in experimental zoölogy during the past few years has done much to extend the list of phenomena, commonly called vital, which are to be accounted for upon the basis of physics and chemistry.

Metabolism. The term *metabolism* includes all those processes involved in the building up of protoplasm and its destruction incidental

to the manifestation of vital phenomena. It includes all the chemical phenomena which are continually going on in the living substance, by which energy is provided for the vital processes and activities, and by which new material is provided for the repair of wastes or for the growth of cells.

Metabolism may be either constructive (anabolism) or destructive (catabolism). In anabolism the chemical processes are in the main synthetic, resulting in the building up of complex substances which finally become a part of the living organism. In the catabolic processes there is a gradual breaking down of the more complex into the more simple substances, frequently through oxidation, and with the liberation of energy. The upbuilding and the down-breaking processes occur at the same time, but one set of processes may predominate over the other to such an extent as almost to obscure it. In general, plants are considered anabolic in their nature and animals catabolic. This means that the plants are able to build up complex living matter out of the raw materials, while the animal is forced for the most part to live upon highly organized food materials, and has preponderant power to break down complex substances.

Rhythmicity.—One property of protoplasm, less conspicuous in certain of its manifestations than some of the others mentioned, is rhythmicity. By this is meant the tendency of the fundamental, or even incidental, operations of the cells of the animal body or of the organism to alternate or oscillate from one side to the other of certain mean values. Some of these oscillations take place very rapidly, others very slowly. In some, one phase of the oscillation may be much more readily recognized than the other. In some, the position of equilibrium may be much farther toward one side than the other. Examples of such fluctuations are seen in the alternation of activity and repose; of contraction and relaxation; of wakefulness and sleep; of accelerated and retarded respiratory movements and heart movements; of the accumulation and discharge of gland cells; of raised and lowered body temperatures; of fatigue and recovery. It is undoubtedly true that this rhythmicity represents fluctuations in the fundamental metabolism of the cells of the animal body. That such fluctuations do occur seems to be established beyond question. Even among the protozoa, where it might be natural to expect to find an unvaried existence, there are evidences of fluctuation, as shown so well by Woodruff in his studies on the reproduction of Paramœcium. Such fluctuations may be the result of alternations in the physical character of the colloidal system—protoplasm—under the influence of variations in the saline balance.

Maintenance of High Temperature.—It has frequently been stated that the power to maintain a temperature somewhat above the temperature of the surroundings is one of the fundamental properties or charac-

teristics of living matter. It is perfectly true that the higher animals do maintain temperatures above that of their surroundings. This is because of the power of these animals to regulate either the rate of heat production in the body, or the rate at which heat may be lost from the body. The lower animals and the plants do not have any means of controlling the rate at which heat may be given off from the body. They are heat producers just as truly as the higher animals, but on a smaller scale, and they dissipate the heat as it is produced. They thus come to have about the same temperature as the environment in which they are placed. In the course of their heat production they may succeed in raising to a certain extent the temperature of their environment.

Growth.—Closely associated with metabolism is the property of growth. Whenever the anabolic processes of a cell exceed the catabolic processes there is an accumulation of material. If this material is living protoplasm rather than inert matter the cell is said to be growing. Mere increase in size may not indicate growth, but swelling.

Consciousness and Memory.—Included in this list of the fundamental properties of living matter are two which have not commonly been so considered. If it is held that the higher animal organism is simply the result of an evolution from a lower preexisting form, and that the real difference between the higher and the lower consists in the degree to which certain properties are developed and not in the fundamental kinds of work which are carried on by the cells composing the body of the animal, it seems necessary to include consciousness and memory as two of the fundamental properties of protoplasm. No one will question the fact that consciousness of existence occurs in the higher animal forms. Animals recognize themselves as different from their surroundings. They apparently recognize themselves as different from other animal forms which are about them. It seems not at all unlikely that many of the lower animal forms also have the power to make a similar distinction. The Amœba recognizes the existence of particles outside of itself. The Paramœcium does this and more - it appears to recognize others of the same kind, and some evidence has been presented to show that it has the power of receiving or rejecting certain foods. Whether this apparent recognition and choice is simply a matter of physics and chemistry is still a matter for investigation.

With the impinging of impulses upon the surface of the organism there come to be modifications of the protoplasm. It may be said that the first action is of necessity upon the plasma membrane and that the result of a change in the plasma membrane is the immediate cause of changes which take place in the interior of the cell. After any given stimulations the protoplasm of the cell is not the same as it was just before the stimulation. It is generally conceded that with nerve cells the result

of the impinging of such impulses is a more or less permanent change in the protoplasm of the cell.

In support of the view that consciousness should be included as fundamental properties of protoplasm it may be mentioned that the same idea has occurred to others. Professor Minot says: "A frank, unbiased study of consciousness must convince every biologist that it is one of the fundamental phenomena of at least animal life, if not, as is quite possible, of all life." And G. F. Stout says: "Wherever there is any kind of feeling, there consciousness exists, and wherever consciousness exists, mind exists."

Reproduction.—One of the most fundamental of all the physiological properties of living matter is reproduction. Without this property any protoplasmic line would cease to exist—with it any protoplasmic line acquires through time a continuity which would be impossible for any individual representative of that line. This continuity of the racial line appears to be the important end. Individuals of any line grow old, or deteriorate in their metabolic processes to such an extent that a reorganization of the protoplasmic material is necessary. This necessity may arise from the using up of essential cell constituents or from the accumulation of not easily eliminated waste matters.

CHAPTER VI

THE CELL

Present conceptions concerning the ultimate structure of all animals and plants come from the work of two investigators of the earlier half of the nineteenth century. Schleiden, a botanist, and Schwann, a zoölogist, in 1838 and 1839, respectively, published papers which laid the foundations of our present *cell theory*. It is now known that all animals and plants are composed of certain structural protoplasmic units termed *cells*, and that they become adapted to the particular work which they have to perform through various chemical and physical differentiations, which are such as to enable them to do their work in an improved manner. This was expressed in Virchow's dictum, "*Omnis cellula e cellula.*"

Elementary Cell Structure.—Even the simplest forms of cells as they are known today are exceedingly complex structures, and it must be assumed that their ancestors have gone through a very long period of evolutionary change to bring them to their present stage of development. The term *cell* has come through usage to mean a small body of protoplasm, containing a nucleus or nuclear material, and surrounded by a cell membrane. It is not necessary in this place to trace the steps which have led to our present understanding of what a cell is. From what has already been said it will appear that the cell is considered as the resultant of a long line of evolutionary change, and that what is seen today and called a cell is without doubt a great advance over the simple elementary thing which first made its appearance as an independent protoplasmic body.

The Nucleus.—At or near the center of the cell lies a highly differentiated body, the nucleus. The differentiation of this body was very likely one of the early steps in the evolution of the cell. In fact, certain cells are known in which there is no definite nucleus, but only scattered particles of rather poorly differentiated nuclear material. The protoplasm of which the nucleus is composed is of a somewhat different quality from that comprising the remainder of the cell. It is a denser gel and may well be thought to have special duties to perform in the life of the cell. The nucleus is not a homogeneous mass, but is composed of a variety of substances combined in a most complex manner. Certain very dense gels, the chromosomes or chromatic material lying in the nucleus, are undoubtedly very complex protein substances, and are now

believed to be the bearers of hereditary tendencies. From the ways in which they react within the cells and in controlling the development of the organism and in transmitting characters from one generation to the next, it is evident that they are not only exceedingly complex but that they are different from each other in the nucleus, and that each such body is a complex of very definite chemical materials of high potency. Less is known about the achromatic material of the nucleus. At the present time it must be confessed that little information exists as to its nature and less as to its significance. About the nucleus and separating it from the cytoplasm of the cell is a very thin membrane. This may be in the nature of a precipitation membrane. It appears structureless, but is undoubtedly a definite structure. From a physiological point of view the nuclear membrane is an important structure, forming a definite separation between the colloids of cytoplasm and nucleus while still providing for the diffusion of oxygen, carbon dioxide, and crystalloids generally. In the more primitive cells, nucleus and cytoplasm may have intermingled, but in all the more highly differentiated cells there is a well-marked boundary. The significance of such a limiting membrane may possibly be indicated at the time of cell division when the membrane is normally broken down and the substances of the nucleus have an opportunity to mingle with those of the cytoplasm. It seems likely that such a mingling at other periods than during cell division might lead to the loss of very important and difficultly replaceable material. The hemming in of this material by a membrane leads to its conservation. The only times when the nuclear material comes in contact with the cytoplasm, which might have a very destructive effect upon it, are the periods of cell multiplication, periods when an equal separation of the material between the two daughter cells is called for.

Functions of the Cell Nucleus.—The work of the nucleus in the daily life of the cell is a matter of importance. A cell without a nucleus may move, respire, digest, and respond to stimuli as would a normal cell. The cell is able without the nucleus to display any activity depending wholly upon catabolic or destructive processes of the protoplasm.

In speaking of the functions of the nucleus a distinction must be made between those functions which have to do with the cell as an individual and those which have to do with the cell as a link in an hereditary chain. In so far as heredity is concerned, the nucleus is the depository of the chromosomes which are believed to transfer the determiners of heredity from one cell generation to the next.

As an organ of the individual cell there is some doubt as to just what the function of the nucleus may be. It appears to be demonstrated that processes involving organic synthesis do not take place in cells deprived of nuclei. Hence the conclusion that the nucleus is primarily an organ for the synthesis of new substance. Various attempts have also been made

to show that the nucleus is the oxidizing organ of the cell. In certain cases this supposition appears to have much in its favor; in certain other cases it has been shown that processes of reduction take place most rapidly in the region of the nucleus.

The Cytoplasm.—The cytoplasm of the cell, that portion of the protoplasm which lies outside the nucleus and within the cell membrane, is colloidal material containing more or less water. This colloidal material is exceedingly heterogeneous, and must include a great variety of substances known to cytologists and designated under such terms as granules, mitochondria, Golgi apparatus, etc. etc., the exact functions of which are entirely unknown. Chambers' account of the structure of the cytoplasm of the living cell, while not entirely complete and satisfactory in all details, gives the known essential facts. In addition to the living protoplasm non-living materials are frequently found in the cytoplasm. Such are oil drops, crystals, granules of pigments, waste materials following metabolism, etc. Frequently a layer of the cytoplasm just beneath the cell membrane is modified into a much more solid and resistant gel, termed the *ectoplasm*. This modification is evidently a cellular adaptation resulting in increased stiffness and mechanical stability.

The Cell Membrane.—About every living cell, and forming a boundary between it and other cells or its environing liquid, is a thin and apparently structureless membrane. In many cases the membrane is exceedingly thin—in fact, it is so thin in some cells that its existence has been denied. In other cells the presence of the membrane is easily detected by the microscope. The membranes about cells appear similar in origin to those which form on the exposed surface of such foams as beaten white of egg, in which there is an actual gelation of albumen particles in the surface layer. The substances which enter into the composition of the films of living cells come from the protoplasm of the cells (and possibly from the liquid surrounding the cells, since the membrane is an interfacial structure) and appear chiefly lipoidal and protein in character. The two types of substances found in the cells which would tend to collect in the surface layers of the cells because of their tendency to lower surface tension are lipoids and proteins. Inasmuch as the protoplasms of different cells may differ chemically in composition, it follows that the composition of the membranes may differ also.

The function of the cell membrane is apparently to maintain a separation between the substances composing the protoplasm and the substances outside of the cell. Whatever substances enter the cell or leave it must do so by diffusion through the membrane. The integrity of the membrane is, therefore, responsible for holding together in a common system the colloids of the cell, and probably also for regulating the inflow and outflow of crystalloidal substances between the protoplasm and its envi-

ronment. The permeability of the membrane varies from time to time according to external and internal conditions. This matter is discussed in another connection.

Cellular Differentiation.—Cells assume, both among the protozoa and the metazoa, the most varied forms and perform the most diverse functions. In all cases, however, no matter how divergent they may become from the typical form, it is still possible to recognize cytoplasm and nuclear material, which may or may not be aggregated into a definite nucleus. The variations which different types of cells present give visible expression of the principle of the *division of labor*. In this differentiation it is very likely that one of the first steps was the distinction of the nucleus or nuclear material from the cytoplasm.

Adaptation of Form to Function.—The forms which cells have come to assume are doubtless determined according to the functions they are to perform. Among the simplest forms of life, where all the vital processes of the organism are bound up within the limits of a single cell, there can be no doubt that the cell is the unit of function as well as of structure. Each cell lives by itself, has its own independent existence, and carries on all the fundamental functions that pertain to any animal. Finally, through a division of its substance, it gives rise to new individuals.

Interactions of Cells.—In forms higher in the animal scale, with the setting aside of certain cells in the animal body for the doing of certain specific things there is a relinquishment by each and every cell of some part of its inherited capacities and functions. This giving up of something is associated with a definite gain in effectiveness in its work along some special line. As a result, there comes to be an interaction, and an interrelation, between the cells which are associated in an organism, so that ultimately the cell ceases to be the functional unit and the organism becomes the unit of existence.

In considering the matter of the physiological differentiation of cells and the morphological differentiation which of necessity follows, it is necessary to keep in mind the fact that, while the cell is becoming modified to do a certain type of work with a higher degree of efficiency, it is still considered a protoplasmic cell.

Special and General Functions of Cells.—The special function which a cell seeks to emphasize does not at all relieve it from the necessity of keeping up its protoplasmic structure. On the contrary, it becomes even more important that the particular mechanism of the particular type of cell should be kept in best possible condition. The cell must still carry on those ordinary metabolic processes essential to keeping a protoplasmic cell in repair. To be sure, the metabolic processes become somewhat modified in character. Metabolism must go on, and go along special lines, and frequently at a more rapid rate than in the undifferentiated cell. These metabolic processes, common to all living

cells alike, may be referred to as the general or vegetative functions of cells.

Among the protozoa, all the functions and properties common to animal cells are carried on within the bounds of a single cell. In such a cell the various properties may be exhibited to about the same degree, or it may be found that on account of some peculiarity of mode of life some one or other function or property of the cell may be developed to a greater degree, without, however, seriously interfering with the other functions of the cell. Where all of the functions of the organism are carried on by a single cell they cannot all be carried on so well as where there is some degree of division of labor. That is, if the cell must turn itself to the task of getting food, getting rid of wastes, moving about for the purpose of obtaining a better environment, or warding off the attacks of enemies, it cannot do all these things effectively.

When animals higher up in the animal kingdom are examined it is found that certain cells specialize to do certain things in the colony of cells of which they are parts. One may be a contractile cell. If so, its structure and energies will be turned in that direction. It will be so constituted as always to be ready for contraction when stimulated. It will need to be extremely sensitive, so as to contract upon the slightest stimulation. And so as to be able to do this work to best advantage for the colony of which it is a part, it will have furnished to it in a form ready for immediate use the food materials necessary for maintaining its structure and for furnishing the energy for its work. Its wastes will be removed from it as rapidly as formed, and fresh supplies of oxygen will be brought to it. It, in its turn, through the fact that it has only the one principal function to perform—that of contraction—will come to contract more quickly, through a greater distance, and more strongly than would another cell not specially differentiated for the purpose. As it is a member of a colony of cells, it will do this work for the benefit of the colony rather than for itself. It has given up something of its own powers, but at the same time has made itself more of a power than it ever could have become if it had remained alone as an isolated individual. In thus putting itself and its power of contraction at the disposal of the community of which it is a member, it gives up its power of spontaneous contraction—or so adjusts itself that it does not normally contract unless stimulated, under the conditions of the environment furnished by the organism as a whole. The cell thus becomes a servant in the community, does not contract unless called upon, and upon demand contracts to the full extent of its ability.

The same general line of thought may be pursued in regard to any other class of cells in the animal body. In each it will be discovered that for each bit of individual activity which the cell has sacrificed there has been a corresponding gain along some other line, so that the cell in the long

run is not the loser but the gainer. At the same time the colony of cells gains greatly, for all the cells of a colony are enabled to do their work with greatly increased efficiency.

Directions of Differentiation.—Upon examination it is found that undifferentiated cells may be modified along one of several different lines according to the type of function which may be facilitated. These are:

1. Modifications tending to facilitate movement.
2. Modifications tending to facilitate secretion, absorption.
3. Modifications tending to facilitate support and protection.
4. Modifications tending to facilitate storage and insulation.
5. Modifications tending to facilitate reception, conduction, and storage of impulses.
6. Modifications tending to facilitate reproduction.

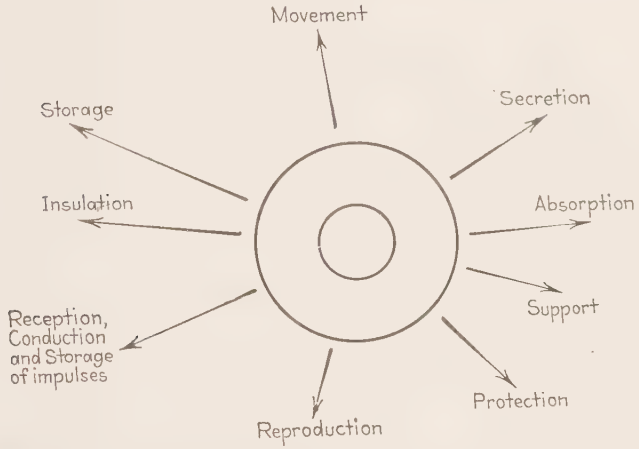


FIG. 9.—Diagram showing lines along which the differentiation of the unspecialized cell takes place.

The accompanying diagram (Fig. 9) indicates in a graphic way some of the possible pathways which may be traveled by a cell in the course of its differentiation. It should be kept in mind that the cell shown at the center is not an egg cell, but one of the undifferentiated, or only slightly differentiated, embryonic cells resulting from early divisions of the fertilized egg.

Cellular Modifications Tending to Facilitate Movement.—One of the most striking properties of living matter is that of movement. Certain forms of cells exhibit a rotation or streaming of the protoplasm within the cell membrane for which there is no evident mechanical cause. Many plant cells and some protozoan cells show this property very clearly, while many other types of cells have a protoplasm which appears very stable.

Very many animal cells are adapted for the display of movement. These adaptations are usually of a permanent character, though there are certain cases in which they are temporary. Among these adaptations may be mentioned pseudopodium-like processes, flagellum-like structures, flagella, cilia, undulating membranes, musculoepithelium, and muscle cells.

1. *Pseudopodium-like Processes.*—These are bud-like projections from the surfaces of cells, produced perhaps through a general contraction of the protoplasmic mass and a weakening of the surface layer on one side of the cell so that a portion of the protoplasm is squeezed out upon that side. There is little if any previous structural differentiation, the cell being capable of forming these projections at any point upon its surface. The formation of the pseudopodia has been accounted for in a variety of ways, and especially has the action of surface tension been stated to account for the facts observed. It appears, however, that surface tensions are of doubtful value in accounting for the formation of these buds unless one restricts the action to intracellular surfaces. Certainly, changes of tension at the cell-water surface can hardly account for the movements observed. It is entirely possible to account for the facts observed if one assumes a variety of interfaces within the cell along which there may be increases or decreases of tension. Such changes would result in protoplasmic movements of considerable amplitude and intensity.

2. *Flagellum-like Structures.*—A second group of structures favoring movement has been observed in certain of the Myxomycetes in which pseudopodia, formed apparently as similar structures in other protoplasmic cells, become converted into more or less permanent structures of a *flagellum-like* form.

3. *Flagella.*—In many of the protozoa and in higher forms as well, there are found long whip-like structures extending outward from the cell, and these by their continual lashing in the water serve as locomotor or food-gathering organs. They are permanent structures. There is little definite information as to their actual method of operation.

4. *Cilia.*—The surfaces of many protozoa and the free surfaces of many of the exposed cells of the higher forms are covered with minute, hair-like processes which, by their lashings in the water in definite ways, serve as locomotor organs or as structures for keeping in motion the liquid which bathes the surface of the cell.

5. *Undulating Membrane.*—Certain cells, notably some sperm cells, are provided with a long narrow membrane capable of a wavy movement which assists in locomotion.

6. *Musculoepithelium.*—In certain of the lower metazoa are cells which act at the same time as sensory, covering, and motor cells. They are frequently of an L or T shape, having a long basal portion, which is

very contractile, and arising from this a slender branch which acts as a receptor of stimuli.

7. *Muscle Cells.*—These are cells in which the whole or a greater part of the protoplasm has become converted into a definite contractile mechanism. There is a great gradation in the degree of complexity of these cells and a great difference in the degree to which the contractile function has been developed. Some, such as the wing muscles of insects, act with great rapidity; others, as the aductors of molluses, act very slowly.

Cellular Modifications Tending to Facilitate Secretion and Absorption.—Much of the work of the organism is of a chemical character. Where all the functions of the organism are carried on by a single cell this chemical work is, of necessity, all done by the one bit of protoplasm. This work involves the production of all the digestive ferments and such other substances as are needed by the cell in its life processes, and not obtained as a part of the food supply.

Cells which are set apart for this chemical work of secretion frequently show little morphological change from the more generalized types of cells. In form, they may approach very closely the typical form. The character of the protoplasm may appear modified by the presence of granules or masses of substance stored up or elaborated by the protoplasm as a part of its special work. The chemical substances produced may be enzymes or their precursors, hormones or chalones, drugs, toxins, antitoxins, etc. It should be understood that the formation of these chemical substances which have been mentioned may occur in other than secreting cells, but the chemical processes leading to the elaboration of the substances in question take place to a greater degree in secreting cells than in other cells of the animal body.

Cellular Modifications Tending to Facilitate Reception, Storage, and Conduction of Impulses.—The property of irritability is one of the most striking of the fundamental properties of protoplasm. The living substance responds to external stimulation by movements or other types of response which are in kind and intensity wholly independent of the amount of energy involved in the stimulus. For example, the stimulus conveyed to the muscle cell is entirely disproportionate to the amount of work which may be called forth as the result of stimulation.

The cellular modifications which make this type of work possible are probably both physical and chemical. The very characteristic forms which nerve cells assume are recognized, with the very much elongated processes reaching from the cell bodies out to some relatively distant portion of the body, and through their processes placing two distant parts of the organism in almost direct physical communication. The long line of ancestral history back of these highly complex structures is yet to be unraveled. Some of the steps which have been covered in the

evolution of these cells can only be guessed at. Professor Parker's studies will doubtless help to make clear some of the phases of the evolution.

A part of the modification is certainly chemical in character. The fact that impressions made upon the nerve cells appear to be quite permanent in many cases leads to the belief that at the moment of stimulation some very definite change must take place in the cell, and that this change leaves some form of permanent impress upon the protoplasm of the cell.

Cellular Modifications Tending to Facilitate Reproduction.—Among the simplest forms of animals the reproductive process may be roughly stated to be a simple division of the existing cell so as to give rise to daughter cells which are, in general, duplicates of the parent cell from which they arise.

Among the higher animals certain cells are set aside for the purpose of transferring to a new generation the characters which taken together have comprised the parent. The specialization of the cells takes place along two distinct lines. In the one type of cell a certain amount of storage material is provided which may be called upon to furnish nourishment for the growing embryo, during the period before it is able to take food from its environment, and to furnish the energy necessary to carry it through the various processes involved in transforming the protoplasm of the egg cell into that of a colonial form made up of definite organs arranged in a particular way. The presence of this storage material adds much to the bulk of the reproductive cell, and renders it particularly inert. Cells of another type, the sperm cells, are especially modified to provide for rapid movement from place to place. It is also likely that, in addition to being motile, they are especially sensitive to the presence of certain specific chemical substances which may serve as directive agents toward the egg cell. In this case the cell is an extremely modified one, the head of the spermatozoön representing the nucleus of the undifferentiated cell, the tail of the spermatozoön being the modified cytoplasm, and the middle piece being the centrosome.

Cell Division.—Just as living matter has its origin only in preexisting living matter, so cells are always the direct descendants of other cells. In considering the events which occur in the process of cell division it must be remembered that the cell is a physical system of great complexity. Many accounts of the phenomena occurring in cell division have been written, but almost all of these have been written from the standpoint of persons who have made a series of careful observations upon material which has been fixed and stained—dead material. Cell division is a sign of life, of intense activity, and should be, and some day will be, described as a series of orderly processes, easily explainable in terms understandable by the physicist and the chemist.

The division of the cell into two following a period of growth appears to be an adaptation of the cell looking toward an increase in its surface

in proportion to the mass. Two chief types of the process have been described, the direct, or amitotic, and the indirect, or mitotic, division. While either of these types may accomplish the restoration of the proper ratio of cell surface to cell mass, the direct method is less accurate in its division and distribution of the various elements of the cell to the daughter cells. If it is correct to think of the cells as physicochemical systems of considerable definiteness of composition and architecture, it must be apparent that in cell division some method must be employed which will provide for the equal distribution of important chemical substances between the daughter cells.

It is undoubtedly true that at the beginning of cell division the protoplasm of the cell is in a liquid condition. At this point one must not be confused by the presence of large quantities of yolk or other nonliving matter which may be held within the cell. This material may be dense, lifeless stuff, but the living protoplasm in which this material is embedded is active, fluid, and has been observed over and over again to exhibit amœboid movements and flowings of a character not at all possible to matter in the gel condition.

It should be recalled that the cell is a complex, heterogeneous system of a number of colloidal substances. In order that the functions of the cell may take place in a normal manner, these substances must, in all probability, be present in definite relative proportions. The process of cell division must, then, involve the division of these different colloidal substances and their distribution to the daughter cells.

The first structure in the cell to divide is the centrosome. This structure has been and still is held by some cytologists to be an organ of prime importance in the formation of the aster, and, therefore, in cell division. The centrosome appears sometimes to be located within the nucleus; in other cases it is purely a cytoplasmic structure. In some cells it appears to be a permanent structure; in others it is only temporary. The forces or conditions within the cell which lead to the activity of the centrosome are not known. Neither is the nature of the forces involved in the actual division of the centrosome into two known. It may be said, however, that the appearance of this body as it divides and separates into two is so similar to the general appearance of the dividing nucleus that one is forced to wonder if the underlying principle at work in the two cases is not the same. After division, the two daughter centrosomes migrate through the cytoplasm along arcs of 90 degrees to positions at opposite sides of the nucleus.

During the period of migration of the centrosomes the nuclear membrane (except in many protozoa) is dissolved and allows the nucleoplasm and cytoplasm to mingle. Under ordinary conditions the rupture of the nuclear membrane and the ensuing mingling of the two plasms end in the destruction of the cell. At this period of the cell's life, however,

the mingling of these different plasms is less injurious. Whether this mingling of nucleoplasm and cytoplasm is a matter of fundamental importance in the division of the cell, or merely incidental, is yet to be determined. The fact that it always occurs would appear to lend emphasis to the suggestion that it is more than incidental in importance.

Among the materials involved in the processes of cell division none is believed to be more important than the nuclear chromatin. In the resting condition of the cell this substance is very inconspicuous in amount and behavior. Staining with various reagents shows that the chromatic material is very irregularly arranged in the nucleus. Injury to the nucleus by a dissecting needle, or the natural instigation of cell division, leads to a most remarkable series of changes in this material. If the nucleus of the dividing cell is like the nuclei of other cells which have been examined, it is a mixture of gels of different degrees of stiffness. The nucleus appears to contain an irregular network with granules embedded in it. The granules are very concentrated masses of gel, the network irregularly disposed masses of a diluter gel, and the interstices the most dilute gel in the nucleus. It is probably true that with the breaking down of the nuclear membrane there is a change in the relative water contents of the different gels of the nucleus. At any rate, the movements which take place during the rearrangements of the nuclear materials can hardly be assumed to take place in gels of high viscosity. The granules which form the densest gel begin to take on the appearance of bead-like swellings strung upon a long thread. This thread of beady granules shows a strongly acid condition, as is indicated by its remarkable affinity for basic dyes. The granules of the thread swell, as would be natural for such colloidal gel material to do in an acid condition, and grow enormously. The separate granules crowd more and more closely together, and in many cases are no longer to be distinguished, even by the highest powers of the microscope, as separate granules. After a time the thread breaks up into a number of segments, known as chromosomes, the number of which appears constant for each species of animals. The granules forming the chromosomes are apparently very important structures of the cell. At any rate, there is evidence that each jelly-like granule in the linear series is divided into two, surface-tension phenomena very likely playing an important rôle in the process. Thus each chromosome becomes doubled.

While these changes are taking place in the nucleus, radical modifications occur in the cytoplasmic portion of the cell. There is formed about each centrosome a structure known from its general appearance as the aster. The two asters together are commonly referred to as the amphiaster, and are connected by a nuclear or cytoplasmic arrangement called the spindle.

The amphiaster comprises two semisolid masses within the more fluid portion of the cell. These structures are the result of a more or less gradual gelation of the cytoplasm immediately surrounding the centrosomes. This gelation spreads until almost all of the liquid portion of the cell is transformed to a stiffer gel. This gel now exhibits behavior similar to that of other gels. It contracts, and as it contracts it forces a separation of liquid from gel; it separates minute droplets of liquid from the continuous gel phase by arranging itself in crystal or spicule forms, leaving droplets of liquid between. As more and more of this liquid is separated from the gel, the droplets run together and form little channels all through the jelly mass. These channels are arranged in a more or less radial manner, with the centrosomes as the centers of radiation. The liquid squeezed out from the gel moves toward the centrosomes and collects about them in the form of liquid spheres, the centrospheres.

The formation of the amphiasters and the mutual pressure of these two masses probably cause the elongation of the cell during cleavage. The division of the cell into two is due to the growth within the cell of these two gelatinous masses at the expense of the liquid cytoplasm. Cleavage occurs when the cytoplasm is nearly entirely incorporated into the two masses. The high surface tension of the equatorial band seems now to indent the surface layer of the cell into a furrow, which becomes deeper and deeper until it finally cuts the cell into two. After the furrow has formed and separated the cells, the semisolid masses revert to a more fluid condition. If the formation of the furrow is prevented by any means, the cell becomes a single semifluid mass containing two nuclei.

The facts as to the division and separation of the chromosomes are well known. Each chromosome becomes split into two entirely similar halves, one of which travels toward each centrosome. Whether the chromosomes are carried along by currents of streaming liquid or by the contractions of colloidal threads attached to them, or in some other way, is yet to be determined.

Up to this point the functions and properties of cells have been considered. Now, turning aside from this consideration, and thinking of the organism as the unit of function, the attempt will be made to determine how the organism does its work. In doing this it will be necessary to make a comparative study of the different processes or functions as they are carried on in a variety of animal forms, beginning with the more generalized and working toward the more specialized. In this way it may be possible to get something of an evolutionary concept—a concept of the advance which has been made in bringing the various processes and systems studied to the high degree of development found in the more complex organisms.

In making this study it must be kept constantly in mind that, for the purpose in hand, the organism dealt with is a physicochemical system;

that the organs and tissues which compose it are physicochemical combinations; that the operations involved in the life processes are physical and chemical processes; and that the laws which govern and control the workings of the inconceivably intricate system are the laws of physics and chemistry.

CHAPTER VII

THE GENERAL PHENOMENA OF LIFE

The characteristics which are held to be a diagnostic for living matter have already been stated. Instead of considering the substance which forms the basis for the vital phenomena, the phenomena themselves may be considered. In other words, an attempt will be made to get away from the idea of structure as the fundamental thing and think in terms of function. The phenomena which pertain to living things may be classed under three general heads, metabolism, energy transformation, and changes of form.

1. Metabolism.—In this term is included the sum total of the changes of substance of the living organism. The cells of which the living organism is composed are constantly undergoing change in a chemical way. These changes are brought about in the processes of digestion, nutrition, respiration, circulation, secretion, and excretion.

2. Energy Transformation.—All of the substances taken in by the cell contain potential energy which can be and is transformed in the living organism to kinetic energy and appears as motion, light, heat, or electricity.

3. Changes of Form.—The constant modification of the protoplasm through the taking in of food leads to a gradual increase of mass on the part of the cell or the organism. In many forms it leads to the development of a more complex structure out of a more simple one as it comes to adult life, and finally to the setting aside of a cell or cells which, in turn, will go through the same cycle as the cells from which it arose. This leads to what is called reproduction.

Conditions Essential for the Production and Maintenance of Life.—Living matter must be considered a result of the evolution of matter. As the forms which non-living matter assumes are considered and it is remembered that all the elements are made up of similar units, the electrons, combined in varied numbers and arrangements, and that the inorganic compounds are simply combinations of these elements, it must be apparent also that living matter is but another manifestation of this same power to combine exhibited by the various inorganic elements. The same electrons, the same elements are present, and some of the same simple compounds of the elements of lower atomic weight. There is, however, this difference: The living substance and the compounds derived from it are vastly more complex in structure and arrangement than

any of the so-called inorganic substances. The molecules of the proteins, which comprise a most important part of the living substance, are very large and exceedingly complex in their configuration.

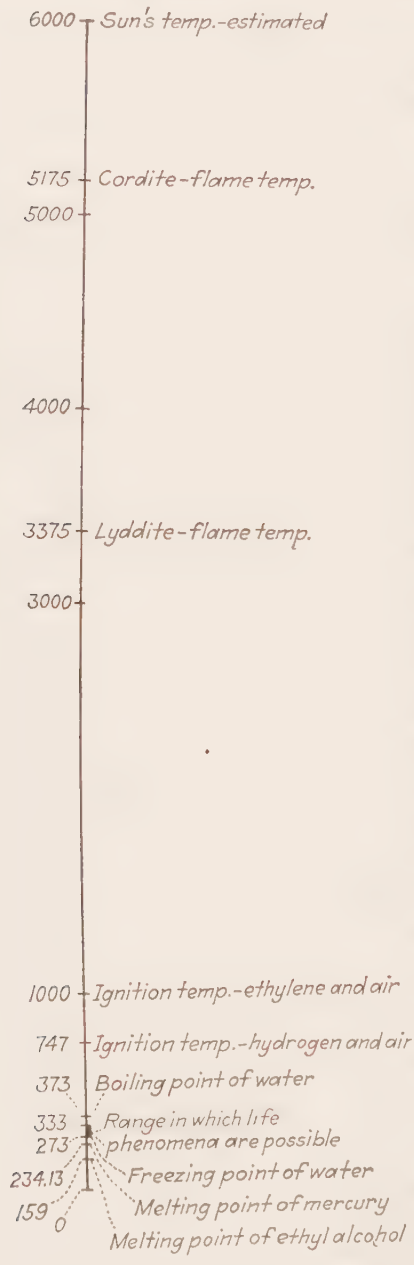


FIG. 10.—Thermometer scale reading from absolute zero to 6000°C. Note the exceedingly limited range in which life phenomena are at all possible.

Factors Conditioning Life.—How life originated upon the earth is not known. Many guesses have been made as to the nature of the conditions which must have existed in order to render the origination of living matter possible. Also many attempts to create living matter in the laboratory have been made. None of these has been successful. The secret of the origin of life is still baffling. And while this is true it seems perfectly evident that there are certain things which can be said as to the conditions upon the earth which tend to make life possible.

1. *Heat.*—When considering the possible range of temperatures from absolute zero up to, say, the temperature of the sun, it becomes evident that the range included between the temperature limits at which life can exist is extremely limited. The temperatures of the earth, land and sea, must have been within these narrow bounds. A lower temperature would bring about the freezing of the water which is so important a constituent of the living substance, and a higher temperature would result in the destruction of the living substance. Also the chemical processes which are characteristic of living matter can apparently take place in a satisfactory manner only within the temperature limits just noted (see Fig. 10).

2. *Light.*—It is possible, though it does not appear probable, that physicochemical systems capable of self-

continuance may have been produced upon the earth while all was dark. It is much more likely that the light appeared first. The formation of the earth was undoubtedly accompanied by light, and while the atmosphere of the earth may for a long time have been too dense to permit easy penetration by the sun's rays, it cannot be doubted that this light energy had an important effect in maintaining life upon the earth. The earliest organisms had no chlorophyll and therefore may have been more dependent upon heat energy than upon light energy. One of the effects of the presence of light was the production of chlorophyll-bearing forms, and these appeared at a comparatively early period. From that time on light has been a most important conditioning factor of organic existence.

3. *Water.*—Water is the chief single constituent of living matter. It comprises more than 80 per cent of all living matter and more than 95 per cent of some. Without it the living substance would be so modified as to fail utterly to do its work. Water is the one universal solvent. Through its aid the chemical and physical operations of the living substance are made possible, as well as the maintenance of its peculiar architecture. The question may arise as to whether some other substance might not be substituted for water in the organic realm. No other substance has yet been found which as a solvent exhibits the properties of diffusion, latent heat, etc., which render water especially important as a constituent of living matter.

4. *Carbon Dioxide.*—There was a time when the earth's atmosphere consisted chiefly of water vapor and carbon dioxide. These elements together formed the source of all the exceedingly large list of complex substances produced by living creatures, and they are the common end products of the breaking down of the constituents of protoplasm.

5. *Oxygen.*—Oxygen is one of the most universally distributed of the elements. It enters into the composition of probably 60 per cent of all inorganic chemical compounds and probably a much larger per cent of organic compounds. Oxygen and hydrogen together hold among the elements an unrivaled power to confer chemical activity wherever they may be. With these elements as the primary factors involved in metabolism and forming an important part of the environment and of the organism, living matter is enabled to make use of other elements as needed.

6. *The Colloidal Condition.*—The colloidal condition of matter must have been realized early in the earth's history. Living matter is not known in any but the colloidal condition, and there is good ground for believing that this condition is essential for the characteristic manifestations which are included under the term *life*. It is this condition of matter which provides for change—for transformation of energy—amidst a continuity of bodily form. It also provides for marvelous chemical and physical complexity of arrangement. It is in such complex struc-

tures that it would be expected that there would be found those chemical and physical instabilities which would result in reactions such as are characteristic of living material.

7. *The Hydrogen Ion.*—Living matter as it appears upon the earth today is much influenced by the reaction of the medium in which it lives. The concentration of the hydrogen ion is known to have a marked influence upon the activities of many forms of cells. It is presumable that during many ages living matter existed in an environment of definite and only slightly changing hydrogen-ion concentration. Whether the living matter might have adjusted itself to some other concentration may be a matter of question, but it seems certain that the existing concentration of the hydrogen ion in the sea is very nearly the optimum for very many forms of life.

8. *Special Conditions.*—The environing conditions are not alike all over the earth. There are variations in the essential conditions which may act upon the living matter. Temperature, the concentration of inorganic substances in the water, the hydrogen-ion concentration, the amount of water available, the character of food supply, etc., etc., vary from place to place. Some types of living matter have become adapted to the limitations of a definite and constant environment. Others have become adapted to some other environment. To make a sudden change from one environmental condition to another might prove fatal—but it may be possible to change from one to another slowly and by slight gradations.

Origin of Living Matter.—There is, of course, no knowledge as to the exact time or method of the formation of living matter on the earth. Ideas regarding this origin must be controlled in a measure by ideas of the origin and formation of matter, of the solar system, etc. There can be no doubt that the existence of matter in the colloidal state is, as Graham stated many years ago, associated with mutability and change. Colloids are in a dynamic state as opposed to the static state of crystalloids.

Benjamin Moore has made a carefully conducted series of experiments to determine whether the colloidal condition of matter can have played any important part in the origin of living matter. It is not possible to discuss his experiments in detail, but certain of his results can be stated. He holds definitely to the idea that there is a type of energy, *biotic energy*, which he considers the energy of life in the bodies of living animals and plants. Biotic energy, if his idea be accepted—life processes, whether it be accepted or not—is primarily dependent upon the energy of sunlight. The fanciful idea of spontaneous generation, which provided for the appearance of organisms out of masses of decaying organic material, was long ago cast aside. No way was found to account for the first appearance of organic material, and yet at some time it appeared. Moore suggests a type of spontaneous generation of living

matter which goes back to the very beginnings of life upon the globe.

The first point to be emphasized in this connection is the fact that temperature is a factor which determines the complexity of chemical materials upon the earth. At the highest temperatures only individual elements can exist. At somewhat lower temperatures the oxides are able to exist. Lower still the saline compounds of the halogens and the carbonates can exist. It is only between 0 and 60°C. that many of the highly complex substances characteristic of living matter are able to exist in a normal condition. These temperatures permit the aggregation of molecules not only into the form of complex substances, but also the molecules of some single substances become aggregated so as to form colloidal particles. The unit of structure in these cases has passed from the electron to the atom, and from the atom to the molecule. As the size of the unit of structure increases, the rapidity of movement of the unit decreases, so that there comes to be a condition in which the large molecules are in equilibrium with their environment. It is possible to imagine that with the increasing complexity of structure the equilibrium in the colloidal aggregates would approach the readily changing condition which is recognized as characteristic of living things. Moore suggests that as the complexity of structure increased, the nature of the equilibrium in the colloidal aggregates would more and more approximate to that labile, easily destroyed, but also readily constructive condition which has been described as characteristic of all living things. So it is possible that the gap between the living and the non-living can be bridged over, and a new idea of spontaneous generation is established, but occurring at a level far below anything of the sort previously suggested among the inorganic colloids. And it must not be thought that this type of generation was an event which took place millions of years ago, never again to be repeated, but rather that it has been a continuous process. Moore says:

> In this process of chemical evolution up to the stage which we at the present day regard as living, it is to be observed that as the non-living colloid becomes more and more complex, and accordingly more and more labile, it at the same time must become more and more susceptible to the influence of external stimulation by different forms of energy. This means that it must, under such conditions that it can remain in equilibrium without decomposition, become more and more a machine for uptake and utilization of energy. The fact that the present basis of the system of living creatures in the world is light energy leads to the view that at a certain stage in the development of colloids, probably long before the appearance of chlorophyll, the colloids began to be affected by light, and acquired the property of retaining and utilizing light energy for further development of structure, or, in other words, synthesis of more complex colloids.

A second point of importance is the gradual increase in time periods arising as development progressed. The simpler a chemical reaction is, as a general

rule, the more instantaneous it is; with increasing complexity the time phases grow longer. This, as stated previously, Graham observed in regard to colloids which are infinitely slower in their reactions than crystalloids.

The earlier stages in development up towards life would hence be rushed through rapidly and be easily and constantly reproducible. As complexity increased so would the time grow longer for any chemical evolution. A vastly increased time interval would be necessary for the evolution of a course of events from the commencing simpler organic conditions upwards in increasing complexity, and more especially the time necessary to reach any higher stage of organization *de novo* would be immensely increased on account of instability and tendency to break down again as different and more delicately balanced stages were passed. There would come in here the tendency of external conditions to stop the process at certain levels, and the steeper slopes of development would only be infrequently ascended until the establishment of something resembling a species, but still short of life, arose by a run over into a stable condition in one case out of many possible millions. This species of highly organized colloid could then more easily reproduce itself by inoculation into suitable material than by starting from simpler bodies *ab initio*, and would form a fresh starting point for further development, just as at the stage of living things each new species would form a fresh point of departure. A labile equilibrium, stable and capable of reproducing itself so long as there existed examples of itself with suitable conditions of material and energetic environment, would so be set up, but would require a long period of time for redevelopment if once the type were lost.

In any search for synthesis of colloids up towards living structures we must accordingly turn to a new field, and begin searching for colloids susceptible to light or other forms of external energy, and look for evidence of building up in colloidal complexity and colloidal synthesis under input of energy.

In order to test his hypothesis Moore has made a series of carefully conducted experiments, as the results of which he found that organic matter (aldehyde) could be synthesized from inorganic colloidal uranic and ferric hydroxides in very dilute solution. These colloids are capable of acting as catalysts for light energy, converting it into chemical energy in a reduction process similar to the first stage of synthesis of organic from inorganic substances in the green plant by the agency of chlorophyll. Such a synthesis as this is looked upon by Moore as probably the first step in the origin of life. The process of evolution of simple organic substances having been once begun it seems probable that substances of more and more complex organic nature would arise from these with additional uptake of energy.

Later, organic colloids would be formed, possessing metastable properties, and these would begin to show the properties possessed by living matter of balanced equilibrium, and up and down energy transformations, following variations in environment.

Moore found also that:

> . . . dilute solutions of nitrates exposed either to sunlight or to a source of light rich in light energy of short wave lengths undergoes conversion of nitrates to nitrites. Rain water which has been standing for some time contains nitrates, but no nitrites. If, however, such water is exposed to bright sunlight or ultraviolet light for some time, strong reactions for nitrites can be obtained. Freshly collected rain water or dew always contains a mixture of nitrates and nitrites. These come undoubtedly from the air. Distilled water which has air bubbled through it for some time will be found to contain both forms of oxides of nitrogen. These substances may be formed through the action of sunlight in the upper regions of the atmosphere upon air and water vapor.

Moore attributes these actions to condensations of the reacting substances upon the colloidal interfaces. He calls attention to the fact that the primeval living thing, like the inorganic colloidal systems which had preceded them, must have possessed the power of fixing carbon and oxygen and of building up these substances into reduced organic compounds with uptake of energy. This power is still possessed by the lowliest type of synthesizing cells existing, namely those of the unicellular algæ. Moore refers to the fact that the earliest products of photosynthesis, such as formaldehyde and methylic alcohol, are highly poisonous to the green cells; but fed to them in sufficiently high dilutions can be used as food in the absence of carbon dioxide. Very marked growths have been obtained with these substances as the sole source or carbon. It was also found that in the absence of all other sources of nitrogen save the elemental nitrogen of the atmosphere, but with abundance of carbon dioxide, the unicellular algæ can fix nitrogen, fix and grow proteins. The rate of fixation and growth is, of course, accelerated if nitrites or oxides of nitrogen are present. These may be supplied from the atmosphere, especially in spring and summer.

Life. It is customary to associate the term *life* with the activities which are most familiar, such as the daily occupations, etc. Upon close examination it is found that the term is not to be limited to the human being alone, but that there are a vast number of animal and plant forms which exhibit phenomena that are to be included under the general term *life*. It is found also that these phenomena are associated only with living matter. Life apart from the living substance is not known. All living bodies are found to exhibit these vital phenomena, and it is the presence of these phenomena which distinguishes living from nonliving matter.

A number of definitions of life have been formulated, none of which tells all that it is desired to include in such a definition. Spencer defines life as "the continual adjustment of internal relations to external condi-

tions." This misses the point, because it omits the fact that life is known only as a quality of and in association with living matter.

Another definition reads:

> Life is the principle of animate corporeal existence; the capacity of an animal or plant for self-preservation and growth by the processes of assimilation and excretion, the permanent cessation of which constitutes death; that state of an animal or plant in which its organs are in actual performance of their functions or are capable of performing their functions, though the performance has not yet begun, or has begun but incompletely; or has been temporarily suspended; vitality.

Still another author defines life as "not the sum of qualities of the chemical elements involved in the composition of protoplasm, but a function of the peculiar architecture of the mixture and then of the high complexity of the compounds contained in the mixture."

It is to be noted that none of the definitions of life is wholly satisfying. This may be in part because so little is still known as to the actual nature of the living substance. The colloidal nature of the living substance has been emphasized because it seems plausible to believe that a thorough understanding of the colloidal condition will teach many things concerning the behavior of living matter. It has been stated that "life is a new factor or a new set of potentialities introduced into inanimate matter. It is a new creation." Whether it be just what is expressed in this quotation from Alexander Findlay, it can be truly said that it is a different manifestation of energy transformations from any which are seen in the inorganic realm. It may be that Findlay is right when he says that: "We cannot explain life in terms of physical science." At least, the attempt should be made.

Mechanistic and Vitalistic Conceptions of Life.—While it is not the purpose here to enter into any philosophical discussion of the relative merits of these two views as to the nature of vital processes, the fact should be borne in mind that investigators of vital phenomena have not agreed in their explanation of these phenomena, and have finally come to be lined up in two more or less hostile camps, which may be termed the *vitalists* and the *mechanists*.

The mechanist looks upon the organism as a physicochemical machine, rather simple in structure and which is controlled by forces acting upon it from without. Being a machine, it must be controlled by definite physical and chemical laws, as are other machines. Under definite internal and external conditions the reactions of the machine are considered definite and predictable, just as the running of any inorganic machine is definite and predictable under fixed internal and external conditions. The machine itself is considered subject to modification through forces acting upon it from without and through metabolic processes. When the machine becomes thus modified its responses are

modified, that is, the machine is now a different machine from that which it was before the modification, and hence must respond in a different way to the external conditions or forces acting upon it.

To the vitalist the organism is a machine plus certain unknown factors. The organism is controlled, in part at least, from within, but is subject to influences and forces outside itself. The reactions of an animal or plant are considered problematical because we are not (and cannot be) in a position to know all of the factors involved in any response to outside stimulation, nor to know all the conditions existing in the machine itself. According to this conception the organism is supposed to be a very complex machine and to involve unknown as well as known forces. Consciously or not, this conception tends to invoke anthropomorphic explanations of behavior of various sorts. Pleasure and pain as experienced by lower animals, for example, are likely to be interpreted in terms of human sensations.

The Duty of the Scientific Observer.—It is the duty of the trained scientist to explain vital phenomena in terms of known physical and chemical materials and laws, in so far as it may be possible to do so; also constantly and consistently to endeavor to extend the explanation of vital activities according to known laws and forces and not to invoke an unknown in order to make an explanation. It is undoubtedly true that scientists have not yet gone as far as may be possible in the analysis of vital processes as the present knowledge of physics and chemistry may make possible. In view of the probability that still greater advances are to be made in physical and chemical research, it seems too soon now to say that some vitalistic explanation of vital phenomena must be relied upon.

The natural tendency of the vitalist is to announce that the mysterious can never be explained. He fails or refuses to use the tools at hand in the effort to carry explanations of natural—and vital—phenomena as far as possible. It should be the task of the vitalist—even more than of the mechanist—to determine the limits of physicochemical explanations of vital phenomena.

CHAPTER VIII

ORGAN SYSTEMS

Living protozoan cells show great diversity of differentiation. Great as this differentiation becomes it does not provide for all the conditions under which such cells may be placed, or provide for all the forms of activity which the cells may be called upon to perform. It appears a fact that as cells become more and more specialized they become less and less fitted to live isolated from each other. Then comes a new stage in organic differentiation in which there are groupings of like cells to form tissues, and of unlike tissues to form more or less specialized organs for the purpose of performing some particular function in the life of the cell group. The very slow and gradual evolution of the various physiological functions is evidenced by the corresponding gradation in the complexity of the various organ systems.

Tissues and Organs.—An organ is commonly defined as a part or structure of an animal or plant adapted for the performance of some particular function or functions. In unicellular animals the organ may be simply a specialized portion of the single cell; in the multicellular animals it is frequently very complex in its structure, being composed of, perhaps, many thousands of cells of different kinds.

In describing the lines along which cellular differentiations have taken place the distinction between tissues of different sorts has been made, for a tissue is simply an aggregate of cells of the same kind, together with their intercellular substance, forming one of the structural materials out of which the body of the animal is built.

It is possible to classify the tissues of the living animal body according to either structure or function. Most textbooks of biology give a structural or morphological classification and say little or nothing as to a functional classification. For the present purpose the latter is especially desirable.

Morphological Classification of Tissues	Physiological Classification of Tissues
	For support
	For protection
Epithelial	For movement
Connective	For secretion
Muscular	For absorption
Nervous	For storage
Germinal	For insulation
	For reception, conduction, and storage of impulses
	For reproduction

A casual inspection will reveal the fact that the so-called morphological classification of tissues and a physiological classification cannot exactly correspond. From a morphological standpoint there are usually considered to be five different kinds of animal tissues. These five kinds perform more than five functions, however, and the work of the different sorts of tissues overlaps in a way that makes a physiological classification of them not entirely satisfactory.

As tissue cells become specialized and gathered into groups or sheets for the performance of some particular function, it becomes necessary that they should have associated with them cells of other types which may aid in making their work more effective. So it is found that motor nerve cells are associated with many secretory and glandular structures in the walls of the alimentary tube and so forth.

And as the activities of the motor cells must be correlated with the demands of the secreting tissue, or the needs of the organism, there must also be associated with them other nervous elements. An organ thus in many cases comes to be a highly complex structure made up of several tissues, all parts of which are closely correlated among themselves and with the organism as a whole. This correlation is of function as well as of structure.

Organ Systems.—The **organ systems** of greatest importance for the organism are the alimentary, circulatory, respiratory, excretory, motor, nervous, and reproductive systems. In addition to these, special devices for protection and defense are found. It must be understood that these systems which in the higher animals appear to be so highly organized and effective in their work were at one time very generalized in their type and work. The increase in complexity and efficiency is the result of a long period of evolution. In the lowest of the metazoan forms, there is little distinction between the different systems they are all poorly differentiated. In the higher animals the differentiation has gone on to such an extent that the character of the work which may be done by any organ system is very closely delimited. For example, among the Cœlenterates the alimentary, circulatory, excretory, and respiratory functions are performed by a common gastrovascular sac. In higher forms each of these functions is performed by an elaborately constituted series of organs.

It is not practicable in such a work as this to consider all the details of structure of the various organ's systems. That is the problem of the morphologist. The student is referred, therefore, to the various texts on zoölogy for a review of the details of organization of specific animal forms. For purposes of hasty review a tabular statement is given here indicating in a very general and sketchy way the course of development of the chief organ systems found in the higher animals.

The Alimentary System. *Protozoa.*—In many cases no permanent alimentary structures. In some there are definite gullets provided with ciliated borders for establishing definite water currents. Digestion takes place in food vacuoles, which are temporary, formed when the food particles are ingested, and which disappear when the food is digested. The undigested residue is eliminated.

Porifera.—Digestion intracellular.

Cœlenterata.—Gastrovascular cavity, simple or branched. Partial contact digestion in gastral cavity. Digestion completed intracellularly.

Platyhelminthes.—Thin-walled tube, leading from muscular pharynx with very many simple or branched, blindly ended diverticula. Digestion partly intracellular.

Nemathelminthes.—Alimentary canal usually well developed, has mouth and anus, and is divided into three regions.

Echinodermata.—Typically a short, broad tube, provided with digestive glands. In some forms the digestive tube becomes elongated and is provided with an elaborate masticatory apparatus, and vent.

Annelida.—Linear tube differentiated into pharynx, œsophagus, crop, gizzard, stomach intestine, with small, pouch-like diverticula. Definite mouth and anus. Sometimes digestive glands are present. Muscle fibers of tract arranged longitudinally and circularly.

Mollusca.—More highly developed apparatus. In some the mouth is provided with a chitinous radula, bearing numerous teeth, and moved by special muscles. Well-developed alimentary tube provided with salivary and hepato-pancreatic glands. In Lamellibranchs gills serve as collectors of small food particles.

Crustacea.—Alimentary tube composed of œsophagus (fore gut), stomach with gastric mill, midgut with evaginated glands which are both secretory and absorptive, and hind gut ending in vent.

Insecta.—Alimentary canal consists of fore gut, midgut with accessory salivary glands, and gastric cœcal pouches. Elaborate mouth parts for sucking and biting.

Urochordata and *Hemichordata.*—Mouth anteriorly, opens into large pharynx, which is perforated by from two to several gill slits, and surrounded to a lesser or greater extent by a peribranchial chamber. This communicates with exterior by atrial pore. Œsophagus opens into glandular stomach. Intestine somewhat coiled, ends in anus. Digestive enzymes similar to those of Molluscs.

Cyclostomata.—Mouth is suctorial. Horny teeth present. Powerful sucker is applied to victim and both flesh and blood are obtained. Rest of gut is straight and simple. A single-lobed liver, but only a hint of pancreas is found. Slight spiral valve in the intestine.

Elasmobranchii.—Mouth a transverse aperture. Teeth numerous on jaws. Nasobuccal groove connects nares with mouth. Stomach is U-shaped, with long left limb passing from œsophagus and a short right limb passing into intestine. Intestine of two parts, large and small. Liver bilobed. Gall bladder in left lobe. Pancreas, rectal gland are also present.

Pisces.—Among the Holocephali are found characteristic teeth, stomach almost obsolete, enteric canal passes in almost straight line from gullet to anus. Well defined spiral valve in intestine. The Teleosts have somewhat more elongated digestive tube, slightly coiled. No salivary glands. No spiracles nor posterior nares. Five pairs of gill clefts open from pharynx. Gill arches bear rakes that strain the food particles from the water and prevent them from being swept out by respiratory current. Gullet, curved stomach, tubular pyloric cœca arising from duodenum, liver, bile duct opens from gall bladder, rectum provided with aperture separate from those of genital and urinary ducts. No pancreas is found.

The Dipnoi have two pairs of large compound teeth, one on roof of mouth, and one on lower jaw. In the enteric canal there is a spiral valve extending throughout the intestine. Rectum opens into small cloaca.

Amphibia.—Many Amphibia provided with protrusible tongue for the capture of food. Lower jaw has small teeth. Mouth opens into pharynx, into which also open nares and eustachian tubes. Alimentary tract long and much coiled. No salivary glands. Pancreas, lobed liver, gall bladder, gastric glands are present. Tract divided into pharynx, œsophagus, stomach (cardiac and pyloric), small intestine, large intestine, cloaca.

Reptilia.—Upper and lower jaws each have a row of small, conical teeth. On floor of mouth is a narrow fleshy tongue, bifid at the front. Salivary glands, poison glands, narrow gullet, stomach cylindrical and little larger than the gullet, short cæcum, bilobed liver, gall bladder, pancreas, coiled intestine.

Aves.—Mouth provided with horny beak; no trace of teeth; pharynx leads into widely distensible gullet which dilates into a large crop just in front of the sternum. Food is macerated in the crop before being passed on to the stomach through the gullet. Gizzard thin-walled in meat-eating birds, thick-walled in grain eaters. Duodenum, cæcal pouches, ileum, rectum, cloaca in three parts. Buccal glands, which are not salivary in function; liver large and divided into two lobes; no gall bladder, pancreas present.

Circulatory Systems. *Protozoa.*—Only circulation is the streaming of the protoplasm within the cell.

Porifera.—System of incurrent and excurrent canals and chambers containing water which is kept in motion by the lashing of flagella.

Cœlenterata.—Common gastrovascular system (simple or complex). Liquid contained within system kept in motion by cilia, flagella, and body movements. Some food substances transported throughout body by phagocytic cells.

Platyhelminthes.—Lymph bathes parenchymatous body cells. No special circulatory or respiratory organs.

Nemathelminthes.—Between enteric canal and body wall is a space called cœlom, filled with a clear fluid. No other vascular system.

Echinodermata.—Vascular system consists of circumoral hæmal tube, with radial hæmal tubes in each ray of animal. No true pulsating heart. Radial tubes frequently communicate with cœlom. Also a water-vascular system composed of circumoral and radial water tubes, ampullæ, etc.

Annelida.—In many forms a double system. Cœlomic space filled with a fluid which washes back and forth between body wall and alimentary canal. This fluid contains food matters and wastes and corpuscles. In many forms a system of tubes containing a red plasma carrying hæmoglobin in solution. Dorsal blood vessel extends nearly the whole length of body, and gives off lateral branches in every segment of the body. Some of these may be much enlarged and serve as pulsating motor organs. This system is a completely closed system of tubes, so as to prevent the loss of hæmoglobin. Its subdivisions extend to all parts of the body.

Crustacea.—Heart tubular, single-chambered, lying in pericardial sinus. Blood enters heart through three pairs of ostia and is forced to different regions of body through five main arteries. Capillaries have open ends and open into sinuses. Blood contains hæmocyanin, which makes blood blue when combined with oxygen. Corpuscles are all colorless leucocytes. Blood returns to heart through sinus, and on its way passes through gills, where its oxygen supply is renewed.

Hexopoda.—Heart, continued in front in aorta, is only part of blood system with definite walls. Heart is an elongated tube closed behind and open in front, runs along middle line of abdomen and thorax just beneath terga. It is in the pericardial sinus, a part of the hæmocœl, as in the crayfish. Inside it is divided by valves opening forward. Walls are perforated by ostia.

Mollusca.—Heart consisting of one or two auricles and ventricle lies in pericardial chamber. Arteries arise from both ends of heart and ramify through body. Many

of them end openly into sinuses, through which the blood returns via kidneys, gills, and vena cava again to auricles of heart. Blood colorless and contains leucocytes. Among the Cephalopoda the system of tubes is more completely closed, sinuses occurring only in the head regions in some forms.

Urochordata and Hemichordata.—Heart simple and tubular, merely a dilation of main artery. Branchiocardiac and cardiovisceral vessels carry blood to gill and visceral structures respectively. Blood then passes through lacunæ and sinuses and returns to heart. Periodic reversal of beat sends blood first to pharyngeal vessels and then to general body vessels.

Cyclostomata.—Blood contains amœboid leucocytes and red blood corpuscles, elliptical in form. It is collected from body in anterior and posterior cardinal veins and passes through sinus venosus into auricle of the heart; then to the ventricle; then to the aorta; aorta gives branches to the respiratory pouches. In the lamprey the system is essentially the same except that the red blood cells are biconcave, circular, nucleated discs.

Elasmobranchii.—Heart of four chambers, sinus venosus, auricle, ventricle, and conus arteriosus, through which blood passes in the order given. From gills the blood passes by efferent branchial arteries. Ventral aorta has a pair of afferent branchial arteries for each branchial pouch. Dorsal aorta has numerous branches. Veins are thin-walled and larger trunks are strongly dilated and called sinuses. There are renal and hepatic systems by which kidney and liver are supplied with blood.

Pisces.—Four-chambered heart consisting of sinus venosus, auricle, ventricle, and conus arteriosus. Heart contains only venous blood. Well-developed closed system of arteries, capillaries, veins. Renal portal system present.

Amphibia.—Heart consists of sinus venosus, right and left auricles, ventricle, conus arteriosus. Well-developed system of arteries, capillaries, and veins. Also well-developed lymphatic system, from which lymph is pumped by lymph hearts into veins.

Reptilia.—Heart lies in pericardial sac; consists of sinus venosus, right and left auricles, and an incompletely divided ventricle. A two-lipped valve leads from sinoauricular aperture to right auricle. Well-differentiated system of arteries, capillaries, and veins is present. Renal portal and hepatic portal systems are present. Lymphatic system with a pair of lymph hearts also occurs.

Aves.—Four-chambered heart. No sinus venosus. Semilunar valves at entrance to aorta and pulmonary artery. Left aortic arch absent in adult. Systemic arteries receive pure arterial blood from left side of heart. Aerated and non-aerated blood mingle only in capillaries. Renal portal system almost obsolete.

Mammalia.—Heart in thoracic cavity, lies between two pleural sacs which enclose lungs. Heart surrounded by pericardial fluid and enclosed in sac. Heart four-chambered. Semilunar valves at entrance of pulmonary artery and aorta. Right auricle separated from right ventricle by tricuspid valve. Left auricle and ventricle by mitral valve. Extensive system of arteries, veins, and capillaries, so that entire body is supplied with blood.

Respiratory System. *Protozoa.*—General body surface serves as chief respiratory organ. Liquid carried in during formation of food vacuole gives a certain amount of oxygen. Liquid thrown off by contractile vacuole carries off some carbon dioxide.

Porifera.—General body surface and exposed surfaces of water canals and chambers serve as respiratory organs.

Cœlenterata.—General body surface and linings of gastrovascular system serve as respiratory surfaces.

Platyhelminthes.—Body surface serves as respiratory organ.

Nemathelminthes.—General body surface serves as respiratory organ.

Echinodermata.—On aboral surfaces respiratory papillæ, lined with ciliated epithelium, and filled with slowly circulating cœlomic fluid. Among the Holothurians the respiratory tree, a diverticulum from the cloaca, is alternately filled and emptied with sea water, and serves as a respiratory organ.

Annelida.—General body surface serves for respiration. Some forms are provided with gills on anterior end or have them segmentally arranged. Some also use parapodia (motor organs) for respiratory purposes. Hæmoglobin the usual respiratory pigment.

Crustacea.—Generally by means of gills which are borne upon the basal segments of thoracic appendages, or higher up on body wall, and contained in gill chamber formed by an extension of the carapace. Among the Isopods conditions intermediate between those characteristic for aquatic and air-breathing forms are found.

Hexapoda.—System of tracheal tubes opening on the surface at the stigmata. They ramify through all parts of the body, branches extending in some cases to individual cells. The tracheæ have a chitinous lining supported by a spirally wound fiber-like thickening called the tænidium. Rapid and complete oxygenation required by insects is thus provided for. Transitional stages between air-breathing and aquatic forms also occur.

Mollusca.—In all aquatic forms gills supported on the sides of the body mass and lying in the mantle chamber serve as respiratory organs. The work of the gills is in part that of food gathering, and the highly vascular mantle shares with the gills the respiratory function.

Urochordata and Hemichordata.—Simple gills on the arches of the pharyngeal wall. Pharynx greatly enlarged to form spacious cavity with perforate walls. Blood circulates through vessels on the framework of the pharyngeal wall and there receives its oxygen and gives up its carbon dioxide.

Cyclostomata.—Gills in Lamprey, instead of opening directly into the enteric canal, in the adult open into a respiratory tube lying below the gullet. It opens from the gullet and ends blindly a short distance from the heart. Seven pairs of gill pouches occur here.

Elasmobranchii.—Organs of respiration are gills located in five gill pouches. Gills are on the anterior and posterior walls of the pouches. Each hemibranch consists of a series of close-set, parallel folds or pleats of highly vascular mucous membrane.

Pisces.—Among the Teleosts the water passes in through the mouth and out through the gill slits, through the branchial chamber under the operculum. Swim bladder may in some cases function as a lung.

Amphibia.—In all larval forms and in adult stages of aquatic forms gills are the chief respiratory organs. Lungs serve for respiration in adult land-living forms. These lungs are sac-like diverticula from the alimentary tract. In addition, the general body surface serves a respiratory function, especially in the case of forms which hibernate in mud.

Reptilia.—The lungs are the chief respiratory organs. These connect with the outside through the bronchi, tracheal tubes, larynx, and buccal cavity.

Aves.—A larynx leads through a partially ossified trachea and bronchial tubes into small lungs. The bronchial tubes connect also with air spaces extending into the bones. Inspired air is thus distributed all over the body so that the aeration of the blood is not confined to the somewhat limited lung surfaces.

Mammalia.—Highly specialized internal lungs, outgrowths from the embryonic alimentary tract, terminating in thin-walled alveoli which are richly invested with blood capillaries. Respiratory epithelium of the alveoli is only one cell in thickness. Ventilation of the lung is effected by increasing and decreasing the size of the chest cavity by means of the thoracic muscles.

Circulating Fluid. *Protozoa.*—None, unless water taken in through the wall be considered such.

Porifera.—Water.

Cœlenterata.—Water plus small amounts of food matter in suspension.

Platyhelminthes.—Water diffused through wall of alimentary canal and containing dissolved salts and food and waste matters.

Nemathelminthes.—Cœlomic fluid.

Echinodermata.—Cœlomic and perivisceral fluid surrounding the alimentary tract. Similar fluid in circumoral and radial canals. Water-vascular system contains water and some proteins.

Annelida.—Hæmolymph, sometimes containing a blood pigment and also cœlomic fluid. This is water plus food and waste matter in solution plus corpuscles.

Crustacea.—Blood rich in proteins and other dissolved matters plus amœboid corpuscles of various types. Hæmocyanin is the pigment in most forms, though hæmoglobin is sometimes found.

Hexapoda.—Blood rich in food matters; has little oxygen-carrying power. No respiratory pigment (some exceptions).

Mollusca.—Blood fluid much richer than water; contains proteins, respiratory pigments.

Urochordata and Hemichordata.—Blood containing numerous circular, nucleated discs containing hæmoglobin.

Cyclostomata, Elasmobranchii, Pisces.—Blood contains many oval, nucleated, red corpuscles containing hæmoglobin. Leucocytes and thrombocytes are present. Lymph.

Amphibia.—Fluid plasma in which float nucleated, oval, red corpuscles. White amœboid corpuscles. Blood clots through the formation of fibrin. Lymph circulating through well-developed lymphatic system made up of lymph vessels and immense sinuses. Lymph pumped into veins by two pairs of lymph hearts.

Reptilia.—Blood and lymph.

Aves.—Blood. Red corpuscles are oval, nucleated cells, and contain hæmoglobin. Temperature high, 38°C., or more. Lymph.

Mammalia.—Highly specialized blood in completely closed system of tubes. Red corpuscles are biconcave, non-nucleated discs except in a few cases. Leucocytes and thrombocytes. Blood does not come into actual contact with tissue cells except in lymph glands. Hæmoglobin the oxygen carrier. Fibrinogen in plasma for clotting. Lymph is the immediate environment of the tissue cells.

Excretory System. *Protozoa.*—General body surface plus the contractile vacuole.

Porifera.—No special excretory organs. Wastes carried off by water through canals.

Cœlenterata.—No specialized excretory organs except in certain medusæ. Waste is generally expelled through opening of digestive cavity.

Platyhelminthes.—Two long, much-coiled tubes whose branches end internally in *flame cells* and which open to the exterior through the nephridial pores. Nephridial tubules are intracellular in small forms and intercellular in larger forms.

Nemathelminthes.—Nephridia in general like those of the Platyhelminthes. Among the Acanthocephali there is found a pair of remarkable nephridia with the posterior end near the genital aperture. Each is a system of branching canals, the terminal branches of which, on the terminal lobes of the tree-like nephridium, are provided with ciliary flames. Perforations at the end of each lobe connect with the body cavity.

Echinodermata.—No well-defined excretory organs. Diffusion of waste materials may take place through respiratory papillæ or through respiratory tree. The amœboid cells of the cœlomic fluid serve to carry off wastes through the gills. Waste

matters may be stored in various parts of the organisms. The liquid of the water vascular system contains also a considerable amount of waste material.

Annelida.—Nephridia, bathed by cœlomic fluid, arranged segmentally or in special localities, carry off wastes from the cœlomic fluid. Gland cells along the tubes may have the power to excrete certain dissolved substances. The nephridia frequently are richly supplied with blood vessels.

Crustacea.—At the base of each antenna is an organ of greenish color, the *Green Gland,* by which the function of renal excretion is performed.

Hexapoda.—At the junction of the stomach and intestine a bunch of numerous thread-like yellow appendages, the Malpighian tubules, are the renal organs of the animal.

Mollusca.—Nephridial tubes with glandular walls, leaving from cœlomic or pericardial space to the exterior. Have general capillary supply. The pericardial epithelium is often modified for excretory purposes.

Urochordata and Hemichordata.—Excretory apparatus very slightly developed. No true nephridia, though ciliated funnels do exist in some forms. In some forms the excretory system is represented by a single mass of clear vesicles, without a duct, lying in the second loop of the intestine. In these vesicles are found concretions containing uric acid.

Cyclostomata.—Kidney is a mesonephric, the ureter a pronephric duct. The ureters from the kidneys unite distally in the urinogenital sinus (not in the hag) into which there open two genital pores from the body cavity. The sinus opens like the anus into an integumentary cloacal chamber.

Elasmobranchii.—Each kidney made up of two parts. The anterior part is a long, narrow red ribbon, which runs throughout the greater part of the body cavity, at the sides of the vertebral column. The posterior portion is a small lobulated, dark-red body, lying at the sides of cloaca, at the end of the anterior part. Both have ducts and are covered with peritoneum.

Pisces.—The kidneys are lobed; in male much longer than in the female; mesonephric in origin; composed of masses of true nephric tubules. No true urinary bladder. From posterior part an unpaired ureter extends to aperture, before reaching which it gives off a small bilobed sac, called the bladder.

Amphibia.—Paired mesonephric kidneys lying on dorsal wall of abdomen at sides of vertebral column. Ureters lead from near posterior end of kidney to cloaca. The so-called urinary bladder which projects from ventral side of cloaca is equivalent to an alantois. The kidneys also bear nephrostomes opening into the cœlom.

Reptilia.—Paired kidneys of irregular form, divided into anterior and posterior lobes, open through fine ureters into cloaca through its dorsal wall. Bladder opens into cloaca through its ventral wall.

Aves.—Paired kidneys, each made up of three lobes, metanephric in origin, open into middle portion of cloaca (urodæum) through fine ureters. The embryonic kidney (mesonephros) atrophies early.

Mammalia.—Paired kidneys, lying on dorsal surface of body cavity near vertebral column, metanephric in origin, somewhat compressed, oval in outline with notch, the hilus on inner edge. Hilus is point where uretur and blood vessels join the kidney. Kidney composed of very many tubular units which converge to open into a wide chamber, the pelvis, which forms the dilated beginning of the ureter. Ureter leads backward to urinary bladder.

Nervous System. *Protozoa.*—Certain ciliated and flagellated forms show intracellular differentiations supposed to be nervous in character. Fibrils extend from the center to the bases of cilia and other superficial structures.

Porifera.—No definite receptors or adjustors differentiated; only very primitive effectors.

Cœlenterata.—Beginnings of differentiation of receptors and effectors. In many forms nervous system consists of a diffuse nerve net composed of protoneurones. Nerve cells connect with nematocysts and with muscle cells. Among many Medusæ the nerve net is condensed into a double nerve ring running around margin of bell. Sense organs developed on the margin of the bell.

Platyhelminthes.—Ganglion cells tend to become aggregated in anterior end of body. Sense organs are of a very simple type. Nerve net thinly scattered over body. Among the Turbellaria, nerve cells are aggregated into a pair of ganglia in the anterior region. These give off lateral nerve cords. Among the Trematoda nerves pass forward and backward from nerve centers located near the anterior end of the body. The Cestoda have two or more longitudinal nerve strands and anterior commissures connect the ganglionic masses.

Nemathelminthes.—Nematoda have nerve ring surrounding gullet with six nerves going forward and six backward. In Acanthocephala there is a single large ganglion at the base of proboscis sending off nerves in various directions. In male, there are also two ganglia innervating the reproductive organs. In Chætognatha, the nervous system is more highly developed. Large dorsal brain giving off two œsophageal connectives which unite in a ventral ganglion and give off nerves. Brain sends nerves to eyes and olfactory regions and is also connected with two pairs of ganglia in the head which lie deeply sunk in the mesoderm.

Annelida.—Among the *Polychæta* there is a well-developed nervous system with bilobed cerebral ganglion or brain. From this nerves extend to tentacles to two pairs of eyes. Two œsophageal connectives extend around the pharynx to meet on the ventral side. From these are given off nerves to two anterior pairs of peristomial tentacles. Thick ventral nerve cord provided with a pair of ganglia in each segment from which nerves are given off laterally to various organs. Behind cerebral ganglia is a system of fine nerves with small ganglia, the visceral system, extending to the anterior part of the alimentary canal.

Among the *Oligochæta* the brain and ventral cord are much as above. From the brain nerves are given off to the prostomium and from the ventral cord three pairs of nerves arise in each segment. Stomatogastric nerves pass to anterior part of alimentary canal. No organs of sight or hearing.

Echinodermata.—Nerve net centralized in circumoral ring and radial nerve ending in eye spots at ends of rays in starfish. Radial nerves supply musculature of body and water-vascular system. Cœlomic nervous system also present extending along each ray dorsal to muscles.

Mollusca.—Nerve cells aggregated in cerebral, visceral and pedal—sometimes other—ganglia. Peripheral nerves near ganglia contain few cells, but nearer periphery have more cells. Beneath epithelium a genuine nerve net.

Crustacea.—Dorsal brain united with ventral nerve cord by œsophageal connectives. In some forms ventral nerve chain distinctly ladder-like. In others the longitudinal cords are more or less fused into one. Antennules, antennæ, and eyes supplied by nerves from the brain. First two pairs of ganglia fused with brain proper. A varying degree of condensation and fusion of ganglia occurs. Visceral system coming partly from brain and partly from connectives supplies portions of alimentary tract. Eyes, olfactory organs, and tactile organs occur.

Hexapoda.—Nervous system of ladder type; consists of brain, subœsophageal ganglia, three thoracic and six abdominal ganglia; nerves given off to all parts of body. Also a visceral system.

Urochordata and Hemichordata.—Nervous system very simple. Single ganglion embedded in mantle between incurrent and excurrent siphons is elongated in a dorsoventral direction. Nerves given off at each end.

Cyclostomata.—Nervous system placed dorsally. Brain with very small cerebellum. Optic lobes imperfectly differentiated. Roof of fore brain is non-nervous epithelium. Distinct pineal body with trace of an eye. Spinal cord is flattened; no sympathetic system; the dorsal roots of spinal nerves alternate with ventral roots and do not unite with them to form nerve trunks. There is an external nostril leading into the olfactory sac, a pituitary pouch and a pituitary body, auditory organ with two semicircular canals, taste organs in walls of pharynx.

Elasmobranchii.—Well-differentiated brain. Pituitary body is attached by tubular stalk on its ventral surface to floor of the skull. Midbrain has pair of optic lobes and fiber-like cruri-cerebri. Cerebellum elongated. Surface has few fine grooves. Medulla oblongata, narrow, and continues in spinal cord. Ten pairs of cranial nerves, many pairs of spinal nerves. Eye, ear, and olfactory organs are present. Sympathetic system also present.

Pisces.—Among the Holocephali. Brain: Medulla oblongata produced laterally into large restiform bodies. Diencephalon, long trough-shaped and very thin-walled without pronounced optic thalami; distinct prœncephalon which gives off right and left cerebral hemispheres. Long spindle-shaped olfactory lobes, pineal and pituitary bodies. The Teleostomi have small cerebral hemispheres with defective cortical region, inferior lobes and infundibulum. Large optic lobes, large cerebellum which covers most of the Medulla. Olfactory nerves terminate far from brain. Large optic nerves cross without fusion.

Amphibia.—Brain with five parts—olfactory lobes, thalamencephalon, optic lobes, cerebellum, and medulla oblongata. Ten pairs of cranial nerves, ten pairs of spinal nerves. Sympathetic system has ten pairs of ganglia united by branches to spinal nerves, united to each other by longitudinal trunks and giving branches to heart, aorta, and viscera in pelvic region. Eyes, ears, nostrils, taste papillæ in tongue, touch spots on skin present.

Reptilia.—Brain has all parts, as in brain of frog, with few modifications. Two cerebral hemispheres are oval and narrower in front. Infundibulum ends in pituitary body. Pineal eye present. Anterior commissures, also abberrant commissures. Spinal cord dilates opposite origins of the two pairs of limbs and tapers greatly toward the posterior end of the tail. Nasal cavities have both external and internal nares. Eye present, supported by ring of bone. Ear has two parts, middle and internal; closed by tympanum.

Aves.—Brain completely fills cranial cavity and is remarkable for its short, broad, rounded form. Optic lobes are in lateral instead of dorsal position. Olfactory lobes are very small and olfactory organ is poorly developed. Spinal cord presents large brachial and lumbar enlargements from which the nerves of the fore and hind limbs respectively are given off. Eye has form of biconvex lens. Auditory organ is distinguished from reptiles by the great development of the cochlea. Pecten present in the eye.

Mammalia.—Brain contains same principal parts as that of pigeon, except the cerebral hemispheres are marked by slight convolutions. Sylvian fissure separates off temporal lobe. Large callosum present. Lateral ventricles of cerebral hemispheres much more extensively developed than in the brain of the pigeon. Many more parts and great differentiation. Like most vertebrates there is a sympathetic system. Twelve cranial nerves. Cerebellum very large. Sense organs: eye, pecten absent from it. In ear, cochlea is especially well developed. Nose, etc.

The Reproductive System. *Protozoa.*—No special system of organs. Binary fission, involving both nucleus and cytoplasm; spore formation involving multiple fission. Reorganization of nucleus through process of endomyxis. Incipient sexualism shown in conjugation.

Porifera.—Asexual through budding of sponge colony or through gemmule formation. Sexual reproduction through ova and spermatozoa which arise from amœboid wandering cells of the mesoderm. Ovum develops into a ciliated free-swimming larva which later becomes attached to and develops into sponge body. Sponges are the first successful class of metazoa.

Cœlenterata.—Asexual reproduction through budding and colony formation. Sexual reproduction through ova and spermatozoa (produced in some cases by special reproductive zoöids). Alternation of sexual and asexual generations occurs in many forms. Great variation in the relative importance of the sexual and asexual phases.

Platyhelminthes.—Most are hermaphrodite and many are self-fertilizing. Reproductive organs show some division of labor as in the development of yolk gland, which may have arisen as an overnourished part of the ovary. Among the Trematoda the reproductive system is usually complex. Cross-fertilization occurs, though self-impregnation is more common.

Nemathelminthes.—Sexes usually separate, and organs distinct. Definite sexual dimorphism. In Nematoda the testis in the male is an unpaired, coiled tube differentiating into vas deferens, seminal vesicles, and ejaculatory duct. In the female the ovary consists of paired tubes which pass into oviduct and uterus; vagina on each side. The Chætognatha are hermaphrodite, the ovaries occurring on each side of the trunk region of the cœlom and the testes similarly placed in the tail region.

Annelida.—Hermaphroditic forms: Male organs are testes, seminal vesicles, and vasa deferentia; female organs are ovaries and oviducts. Seminal receptacles are also present. Some forms have the sexes separate. Genital products arise from cœlomic epithelium and are given off to the cœlomic fluid where they complete their development. Spermatozoa and ova discharged by temporary rupture of body wall. Insemination of eggs occurs in sea water.

Echinodermata.—Sexes separate. No sex dimorphism. Sex glands similar in appearance except for color, botryoidal masses, a pair lying in each interradius. Ducts from the glands open to the exterior, usually an aboral surface. Ova and spermatozoa are spawned into sea water, where fertilization and development take place. Naturally, parthenogenesis probably occurs in some forms.

Crustacea.—Diœcious. Definite sexual dimorphism. Abdomen of female broader than that of male. Testes lie in thorax, anterior and posterior lobes. Vas deferens somewhat convoluted. Ovary, three-lobed, branched tube whose epithelium gives rise to ova which pass into lumen of tube, thence through oviducts to the exterior. Males often degenerate. Ova fertilized immediately after laying; develop while fastened to pleopods. Some hermaphrodite or protandrous. Parthenogenesis occurs.

Hexapoda.—Sexes separate. Two testes each with duct leading to seminal vesicles opening into ejaculatory duct. Two ovaries each made up of eight tubes. Each oviduct opens by median aperture on abdomen. Females impregnated by male, fertilization internal.

Mollusca.—Sexes separate or hermaphrodite or protandrous. Gonads large, paired, racemose in Pelecypoda. Short ducts open on surface of visceral mass. Ova pass into cloaca and are fertilized there by spermatozoa. Upon the Cephalapoda, testis is a mass of small tubules, the sperm duct a highly convoluted tube, large sperm sac opening into mantle cavity. Ovary has cavity which is continuous with oviduct and opens into mantle cavity.

Urochordata and Hemichordata.—Hermaphrodite. Ovary and testes simple and closely associated. In Appendicularia, on aboral region of body. Pyrosoma; No gonoducts, ovary contains a single ovum; testes in a diverticulum of peribranchial cavity. Salpa: Ovary has single ovum, ovary and testes closely associated with alimentary canal. Short ducts open into peribranchial cavity. Dolium: Like Salpa, except that ovary has several ova.

Cyclostomata.—Sexes separate. Gonad, elongated, unpaired and moored by a median dorsal mesentery. There are no genital ducts. The ova and the spermatozoa are liberated into the body cavity and pass by two genital and abdominal pores into the urinogenital sinus and then to the exterior.

Elasmobranchii.—Female, single ovary. On surface are round follicles each containing a ovum of bright-yellow color, two oviducts (Müllerian ducts) entirely unconnected with ovary. They unite behind pericardium and open into abdominal cavity. Male, two elongated, lobulated testes attached to wall of abdominal cavity. Several small efferent ducts pass from each testis to the epididymis. Vas deferens runs entire length of non-renal portion of kidney and at end widens into urinogenital sinus.

Pisces.—Holocephali: In male, most mesonephric ducts open into vas deferens, six open into urinogenital sinus. In the female all ducts open into urinary bladder. Female organs much like those of Elasmobranchii and have immense shell glands and uteri. In the male, testes large, ovoid bodies. The Müllerian ducts more fully developed. *Teleostomi:* Testes long, lobed organs. Ovaries are more compact sacs. Two vasa deferentia combine in a single canal. A single oviduct comes from the ovaries. Genital aperture in front of ureter. The *Dipnoi:* Ovaries are long and distinct. Testes also long and compressed and remain distinct. Spermatozoa of male pass out through mesonephric duct. Müllerian ducts in male are very well developed.

Amphibia.—Female has two ovaries which may become exceedingly large; also long convoluted oviducts which widen at the inner end and open near the heart. Wolffian ducts act as ureters here in female. Ova small, numerous, usually pigmented, and with yolk toward one pole. They are almost always laid in water. Usually a metamorphosis in development. Males, a pair of yellow oval testes lying in front of kidney. Ureters serve as sperm ducts.

Reptilia.—All are oviparous. In male, testes are two oval white bodies. The epididymis extends back from the inner side of each and passes into a narrow convoluted tube, the vas deferens, which opens into the terminal part of the corresponding ureter. A pair of vascular evertible copulatory sacs open into the posterior part of the cloaca. Female: Ovaries are a pair of irregular oval bodies situated a little farther back than the testis. Oviducts are thin-walled, wide-plaited tubes which open in front into the body cavity and behind into the posterior end of the cloaca.

Aves.—The testes are ovoid bodies, varying greatly in size according to the season, attached by peritoneum to ventral surface of kidneys. Each sends a convoluted vas deferens to open into the urodæum on the extremity of a small papilla. Posterior end of the sperm duct is slightly enlarged to form a vesicula seminalis. No copulatory organs. Female: Almost complete atrophy of right ovary and oviduct. Left ovary is large and has long convoluted oviduct. Its anterior end has large funnel for catching ova. Tube opens into urodæum. Glands in lining epithelium which give ova its membranes, albumen, and shell.

Mammalia. Rabbit.—Male: Testes, oval bodies, lie in scrotal sac which connects with abdominal cavity by an opening—inguinal canal. A convoluted epididymis close to testes forms procimal part of vasa deferentia. They open into a urinogenital canal into which the urinary bladder is continued. Other parts characterizing the male organs are prostate gland, uterus masculinus, Cowper's glands, perineal glands, and rectal glands, etc. Ovaries, small, ovoid bodies, attached to dorsal wall of abdomen behind the kidneys. Oviducts, very narrow and convoluted, pass into thick-walled uteri, each of which opens into the vagina. Urinogenital canal—passage into which vagina and bladder open; vulva—external opening.

CHAPTER IX

THE TRANSPORT SYSTEM

It has already been shown that because of new demands upon the cells of the body they have come to be differentiated in a variety of ways, and that when they have assumed these differentiated forms they are in a position to be of some special service to the cell community of which they are a part.

The Internal Medium and Its Circulation. When the way in which the individual cells of the body are to get their food supplies and their oxygen supply and to get rid of their wastes is studied, it is found that they have not been able to do very much in the way of modifying the original method of taking in these needed substances through the cell membrane. As will be seen a little later, there has been a modification of a certain sort, but there has not been any elimination of the cell membrane as the organ through which such intake must take place. Protoplasm is a solution. Each cell surrounded by a membrane must have its food substances, etc., brought to it in soluble form, for it is only in this condition that absorption through the membrane can take place. While the fact that the necessity of food and oxygen absorption through the cell membrane has remained unchanged through a long racial history, there has been a decided evolution in the character of the liquid serving as the fluid medium of the animal body. In this particular the animal has shown a marked advance over the plant, for the highest plants even yet make use of the type of internal medium which is to be found only in the more lowly animal organism.

The Need for a Circulating Medium. The need of a special internal medium becomes apparent when the significance of the fact is considered that no new way of getting food or oxygen into the cells except by diffusion through the cell membrane has as yet been elaborated. In any considerable colony of cells it is impossible that all should be superficial in position and thus able to take from their immediate environment the things needed for their upkeep. Nor is it possible to provide a sufficient supply of food or oxygen to the inner-lying cells of a mass because of the constant absorption and consumption of the food materials by the overlying cells.

The protozoa illustrate the simple case in which single cells surrounded by their natural medium, water, in which oxygen is dissolved, and in which minute food particles are floating, move about from place to place

and continually bring their bodies into a new food environment. The more highly organized animal with more cells to feed is frequently much limited in its movements and hence deprived of a surrounding medium as rich as that of the protozoa. At the same time the more highly organized animal with its larger number of cells is in need of greater supplies of food and oxygen than the protozoon would demand. There is a need for much greater efficiency in the distribution of the food matter that is available. As the deeper-lying cells of the organism are the ones especially to suffer from lack of food and oxygen and from the accumulation of wastes, the organisms have devised various methods of bringing these cells into contact with a richer supply than might otherwise be theirs. If the organism is not too large, simple invaginations of the surface may suffice to bring the deeper-lying cells into close association with the external medium. In a great many cases this is insufficient and an internal fluid is employed to bathe the inner- and deeper-lying cells and thus provide them with their needed building materials. Such a fluid may run in more or less definite channels, as blood-vascular systems or lymphatic systems, or may occupy all of the space within the body not occupied by definite organs, and may simply wash about as the movements of the animal determine. The formation of a definite system of tubes, in which a liquid may circulate from one part of the body to another in connection with various invaginate portions, seems to form the best possible arrangement for an efficient distribution of food materials in the animal body. Dahlgren and Kepner have discussed the nature of such systems and have shown, in Figs. 130, 132, 133 of their "Histology," diagrams which will be very instructive in this connection.

Classification of Circulating Media.—The circulating media of various types of animals may be divided and classified as follows:

1. *Hydrolymph*, a watery fluid carrying nutriment to organs and tissues, and removing wastes. This fluid sometimes contains no food matters in solution, though it may hold them in suspension, or in some cases it may contain dissolved proteins or their products. As a rule, it has no respiratory function, though in certain cases the fluid may contain respiratory proteins, and so function as an oxygen carrier.

2. *Hæmolymph*, a circulating medium less watery than the hydrolymph and also containing a richer supply of proteins. It is not only nutritive in function, but also frequently respiratory in character because of the contained respiratory proteins, and corresponds to both blood and lymph of higher organisms.

3. *Blood*, chief circulating fluid of the higher animals. The blood is the most highly differentiated and complex circulating medium found in the animal kingdom and will in the highest forms fulfil all the functions suggested below as applying to blood. Between it and the water found in gastral cavities of sponges and Cœlenterates there exist all possible

gradations of complexity of structure and composition. In the lower forms the fluid is simply a thin, watery solution. In the highest forms the medium takes on the character of a tissue involving the presence of different types of cells embedded in a very fluid and chemically complex matrix or intercellular substance.

4. *Lymph*, a liquid derived from blood through dialysis from the capillary blood vessels, containing white corpuscles, and also proteins and other nutritious substances in solution.

The Functions of Blood.—The blood of any organism serves a variety of functions, and while the functions which are mentioned in this list may not all be found to apply in some particular case, they do apply generally. They are:

1. To carry to every part of the body the materials needed for its sustenance.

2. To carry away from the region of living cells any waste matters.

3. To carry hormones and internal secretions, substances whose function it is to influence coordination of various parts of the organism or to serve as a control of the metabolic processes of various parts of the body.

4. To protect the body in case of invasions by microorganisms.

5. To protect the body by reducing to a minimum the possible loss of blood when the vessels which form the natural channels are injured.

6. To furnish for the tissues of the body a solution containing the salts needed for the protection of the cells of the body in a physiologically balanced condition.

This summary of the functions indicates what is understood to be the functions of the blood of the higher animals. It must be remembered that blood function as well as blood structure has been subject to the evolutionary process. It is doubtless true that in this as in all other parts of the organism there has been a gradual development, and that the blood of the higher animal is functionally, as well as structurally, much more perfect than that of the lower organisms.

The Quantity of Blood in the Body.—The quantity of the circulating fluid in the bodies of various animals varies greatly, depending somewhat upon the natural habit and degree of evolution of the species concerned. In general, it may be said that in those forms in which the circulation is sluggish the proportion of body fluid to body weight is large. This is particularly true in the case of the invertebrated animals. The more active animals, in which there is a more efficient circulation of the blood, require less fluid to meet their needs. The advantage to the animal of being able to reduce the amount of fluid to be carried about, and so the total weight, must be evident.

Various methods have been devised to secure accurate data as to the amount of blood in animal bodies. Welcher's method consists in first bleeding the animal as completely as possible, weighing the quantity

of blood thus obtained, afterwards washing out the blood vessels with water, and estimating the amount of blood contained in the washings from the amount of hæmoglobin contained in them. Grehant and Haldane and Smith have devised methods based upon the power of the blood to absorb carbon monoxide. From the results of determinations upon various animals it is stated that the percentage of body weight represented by the blood is as follows:

	Per Cent
Dog	7.7
Cat	5.0
Rabbit	5.0
Birds	10.0
Man	5.0

In any given species the amount of blood holds fairly constant, but is subject to variations through anemia, obesity, etc.

STRUCTURE OF INTERNAL MEDIA

In order satisfactorily to understand the functions and significance of the bloods of the higher animals one must trace the gradual development of the body fluids from the very simple conditions existing among the lower forms to the highly complex bloods of the higher forms. In the case of the lower metazoa there is simply an appropriation by the organisms of portions of the fluid medium in which they live. This fluid, with little or no modification, serves as a medium of transport for the body. Gradually, as one traces upward along the phyletic series, animals are found which substitute for this rather inadequate circulating medium, fluids elaborated by themselves, more capable of carrying oxygen and carbon dioxide, and containing also many substances useful in the animal body, but which do not appear at all in the body fluids of the lower forms.

Protozoa.—The normal nourishing fluid for the protozoan is the water in which it lives. This may be either fresh, brackish, or salt. This water contains the floating food particles upon which the protozoon feeds, the oxygen necessary for carrying on its body processes, and serves also as a medium for the disposal of wastes.

Metazoa.—In the lower metazoa the water in which the animals live is the medium, with little or no modification, which supplies food and oxygen to the organism and to the cells composing it. This is especially true as concerns the sponges and the Cœlenterata. Among the sponges it is clear that the liquid passing through the incurrent and radial canal systems can be nothing more than water charged with the occasional food particles upon which the animals live. This water is kept in motion by means of flagellated cells lining the cavities of the sponge body, and is taken into the body and passed through and out in a comparatively short

space of time. As the water passes through the canal systems, food particles are taken from it and certain body wastes are added to it. No cellular elements, such as corpuscles, are to be found in it.

Cœlenterata.- Among the Cœlenterata there is to be found a series of conditions somewhat more advanced than among the sponges. No true blood or lymph is to be found. Water is taken into the gastric cavity through the mouth and allowed to circulate slowly and irregularly through the cavities of the body, being sent from place to place by the ordinary movements of the body assisted by the action of certain flagellated cells lining the gastral cavity. The liquid taken in through the mouth is water plus the few bits of food captured by the animal. Few, if any, corpuscles are to be found in the Cœlenterata, though in some of the higher forms free-floating cells have been described. These may or may not be comparable to the corpuscles of higher invertebrates. Many of the cells are well formed and contain nuclei; others are exceedingly rudimentary in appearance. It is undoubtedly true that the process of digestion which takes place in Hydra and many of its relatives is, for the most part, intracellular. Very little, if any, digestion takes place in the gastral sac. Among some of the higher Cœlenterata it appears that the fluid in the gastral sac and circulating though the cavities of the animal is richer in food materials than the water in which they live. It appears to be in some cases a somewhat richer suspension of proteins and other food substances and may occasionally actually contain partially digested matters which wash about in the gastral cavity before being absorbed. The question as to what extent extracellular digestion may take place will be discussed in another place. There is nothing in the nature of fibrin to be found in the circulating fluids of the Cœlenterata and there is no power whatever of clotting. It must be evident that where the circulating fluid is in direct communication with the exterior, as it is in these forms, there can be no opportunity for the building up and maintenance of a richly nutritious vascular fluid.

Echinodermata. Among the Echinodermata there is a marked advance in the nature of the circulatory apparatus and as well in the nature of the circulating fluid. The discussion of the apparatus may well be left until a later time. The fluids circulating in the bodies of the Echinodermata occupy the general body cavity or cœlom, the radial blood vessels, and the water-vascular system. The liquids of the cœlom and the radial blood vessels are in direct communication -really form one solution, which is being constantly enriched by the digested and absorbed food materials diffusing through the walls of the alimentary organs. This fluid also receives oxygen from without through the respiratory papillæ, and through them gives off carbon dioxide to the sea water. The earlier investigators in this field believed that the content of the water-vascular system was simply sea water. More recent studies

have shown that this is not the case, but that the liquid is somewhat more dense, less transparent, and contains a small amount of albuminous material. The liquid is usually colorless, though sometimes colored reddish or yellowish, and contains some amœboid cells. Numerous rounded granules, non-cellular in character, are also found. In some of the Holothurians crystals of calcium carbonate are found in the liquid of the water-vascular system. The body fluid, hydrolymph or blood, of Echinodermata shows a similar content to that stated for the water-vascular system, except that it is somewhat richer in albumin. It is a matter of some difficulty to obtain pure blood from the blood vessels on account of the technical difficulties in the operations involved, but when successful, the blood is usually found to be colorless, though occasionally rose color, yellow or brown, and of a slimy consistency. If one attempts to squeeze blood out of the blood vessels there exudes a brownish fluid, the color of which is apparently derived from the broken cells of the walls of the blood vessels. In Thyone, hæmoglobin occurs in small, very numerous corpuscles.

Worms.—Among those animals commonly grouped under the term worms there is a wonderful variation in the form of the vascular apparatus and such a difference in the fluid found circulating throughout the body that it seems little less than ludicrous to include them all in one group—even for the sake of convenience.

Platyhelminthes.—Among the Platyhelminthes, including the Turbellaria, Trematodes, and Cestodes, there is no definite vascular apparatus. Such circulation of body fluid as occurs consists in the washing back and forth through the parenchyma of the body of small amounts of fluid which has dialyzed through the walls of the gastric cavity. The digestive cavity in its varied ramifications is the nearest approach to a circulatory apparatus, and if the lead which has been given in Cœlenterata is followed, it might be spoken of as a gastral-vascular cavity. In this there is washed back and forth a liquid containing more or less digested food materials, which gradually pass through the walls of the cavity by dialysis and come to bathe, in the form of lymph, the tissues of the general body structure. This fluid coming from the very much-branched gastro-vascular cavity, undoubtedly reaches all the cells of the body.

Nemerteans.—Among the Nemerteans there is found a closed system of vessels, a dorsal and two lateral vessels which unite anteriorly and posteriorly and have also numerous cross-communicating branches. These vessels appear to be the remnants of a cœlom. The blood which occurs in these vessels consists of a colorless plasma in which float a few corpuscles containing nuclei and which may contain hæmoglobin.

Nematohelminthes.—The Nematohelminthes show no vascular system at all. There is a space between the endoderm of the gut and the musculature of the body wall which, while not a true cœlom, contains a clear

fluid derived from the gut and probably acts as blood in these forms. There are few, if any, corpuscles to be found.

Oligochæta and Polychæta.—Among the higher worms, the Oligochæta and the Polychæta, not only are there more elaborate systems for the circulation of the blood than appear in the lower forms, but the fluids themselves are more highly developed. In these higher forms there are two distinct fluids which remain separated. One is the so-called cœlomic fluid, found in the space between the gut and the body wall, and the other is a hæmolymph, found in a series of closed tubes commonly referred to as the blood vessels. There is a very interesting division of labor between these two fluids. The cœlomic fluid receives from the gut the food materials as they diffuse through the walls and conveys these food materials to the various cells of the body. It also receives from the active cells of the body the various metabolic wastes and conveys them to the nephridia, through which they are eliminated. This cœlomic fluid may be said to correspond to the lymph of the higher animals which bathes the individual cells of the body. It contains numerous corpuscles of varying forms. The corpuscles are all unpigmented and may be amœboid cells, cells with numerous, more or less permanent processes developed upon one side, or star-shaped cells.

The fluid in the closed system of tubes is apparently a solution of hæmoglobin. Few, if any, cells occur in this fluid and it seems never to leave the system of tubes in which it is formed. The red fluid corresponds to the red blood corpuscles of the blood of the higher animals and serves as the carrier of oxygen to the various cells and tissues of the body.

Milne-Edwards was much impressed with the lack of colored corpuscles in the blood of the worms and other invertebrates and contended strongly that animals must not be classified as to whether they have red or white blood, but into two main categories: those which have in their blood colored corpuscles, and those which have not. In the main, this distinction agrees with the morphological separation of the invertebrates from the vertebrates. Certain exceptions will be noted. Rouget opposed this form of classification and showed that several classes of animals among the invertebrates possess blood containing colored cells circulating in a closed system of tubes. Among the cases cited are some of the Sipunculid worms, the Cephalopoda and Ascidia. In all fairness it should, of course, be stated that the colored cells found in these forms are hardly to be considered as typical colored corpuscles such as are found among the vertebrates. They are nucleated cells which contain a small amount of colored material in the cytoplasm, more or less collected into a drop or vacuole at one side of the cell.

The color of the blood plasma in the case of the worms is subject to a considerable amount of variation. The red color of the blood of the segmented worms has been known for a long time. Cuvier even tried to

make a classification based upon the presence or absence of red blood. This was later found not to be at all practical. Worms which are comparatively near relatives vary in the color of their blood and it has been found that even within the limits of the same genus there may be a variation of color. Therefore, for the purposes of classification blood color can hardly be considered significant. The reasons for this variation in color will be suggested in the consideration of another group, and while the reasons there offered may not prove to be the only reasons worthy of consideration, they will, it is hoped, at least be suggestive.

Crustacea.—Among the Crustacea, as among the worms, there is great variety in the degree to which the vascular system and vascular elements are developed. In such an account as this it is not necessary to outline the conditions actually existing in any large number of forms, but to point out the conditions existing in a few cases which may be considered typical of the group.

Our understanding of the corpuscles of the crustacean blood goes back to the work of Hewson (1870), who described the colorless amœboid cells which pile up in clumps when the blood clots, and which hold themselves in the clot by means of very long processes.

Later Hardy made interesting observations upon the blood cells of Daphnia. The body of Daphnia is so transparent that it is possible to see clearly and to follow the movement of the blood cells in the living animal. Corresponding to the archaic type of the animal structure, there is in Daphnia no trace of differentiation among the blood cells but all are one and the same type of amœboid cell. Hardy recognized that these cells are exceedingly sticky. At times they are of so clinging a habit that almost all of the corpuscles in an animal will be found to be adhering to the walls of the blood vessels, and almost none in the blood stream. They have the power to crawl about from one place to another, and act in many respects like Amœbæ. If an animal is stimulated there is an aggregation of blood cells at the point of stimulation. A slight local stimulus is sufficient to cause a large number of the amœboid cells to become fixed in the neighborhood of the stimulated part. Here is evidently a very marked display of the property of irritability, the stimulus so far as the corpuscles are concerned being some chemical substance thrown off from the stimulated tissue. This habit of aggregating may possibly be a forerunner of the property of agglutination found very highly developed among animals higher in the animal kingdom.

Hardy also made an interesting observation upon the supposed rôle which the blood cells play in the process of digestion. The yolk of an egg was emulsified with water and by means of a pipette was brought into the bottom of a vessel in which Daphnias were swimming. Most of the animals, through the movements of their appendages, kept themselves supported in the water, but a considerable number sank for a short

time into the emulsion. A careful examination into these individuals which sank to the bottom of the dish revealed the fact that there was a very considerable absorption of fat by the intestinal epithelium, and after 10 to 12 hours a large amount of fat was found in the blood cells. It was also found that when carmine was placed in the bottom of the dish containing the Daphnias there was a considerable absorption of carmine by the lining cells of the intestine, and later the carmine appeared in the blood cells. It has also been noted that in the breeding season there is regularly, in well-nourished individuals, a storing up of fat in the blood cells. This has been explained upon the supposition that the egg production calls for a large amount of fat building on the part of the individual, and that the blood cells act in the capacity of carriers of fat from one portion of the body to another. It appears to the author more likely that the blood cells are acting in this case in the nature of scavengers, and that because of the large amount of fat present in the body they become filled with it as they would with any foreign substance. Incidentally, it might be suggested that Daphnia may furnish an excellent form upon which to work out the mechanics of fat absorption, and to test out the various theories as to how fat digestion and absorption actually take place.

Hardy also made observations upon the blood cells of crayfish, Astacus. In this form he recognized two types of cells both of which appeared to correspond to white cells of vertebrates. One type consists of eosinophil cells whose protoplasm is covered with numerous, usually large, strongly refractive granules. The second type consists of cells which are remarkable for their sensitiveness; they burst upon contact with a foreign body, such, for example as the cover glass. Hardy calls these *explosive corpuscles*. The blood of the crayfish contains about 250 cells per cubic millimeter, of which Hardy estimated the explosive cells to constitute about 75 per cent.

A more recent investigation by Tait shows that in Astacus there are really three types of cells:

1. *Hardy's explosive corpuscles*, which constitute from 40 to 50 per cent of all of the blood cells. They are narrow, elongated spindles, the cytoplasm of which runs out into two pointed extremities. As the cells roll across the field of the microscope they can be seen to be distinctly flattened in one direction, the centrally located nucleus bulging outwards on both sides like the nucleus of the frog's erythrocyte. The protoplasm of the cell is hyaline, and the cell is pale and inconspicuous.

2. *Thigmocytes*.—These constitute from 20 to 25 per cent of the blood cells. They are at the first very much like the explosive corpuscles, but differ from them in the fact that they do not so readily cytolize. After being shed upon a glass slide the thigmocytes quickly suffer extensive changes in appearance. Within a minute they become bluntly oval in

outline, and the surface of the corpuscles becomes studded with small vesicles. From the spaces between the vesicles there are shot out extremely fine pointed processes of clear ectoplasm, which pass directly outward from the cell in contact with the glass. These increase in length and the corpuscle assumes a prickly appearance. The processes continue to move outward and the cytoplasm in the clefts between them also advances so that the whole corpuscle becomes spread out into a very thin film. This film becomes very inconspicuous, and the boundaries of it can be made out only by the most accurate focusing. There seems little doubt that the mechanism involved in this spreading movement is that of capillary attraction. The cell is unstable in contact with the glass, and so expands until the spreading movement is stopped by the internal resistance of the structures of the cell. The thigmocyte has no power to change its position or to retract its protoplasmic processes. The cell does not remain permanently in the condition described, but sooner or later undergoes a definite cytolysis.

3. *Amœbocytes.*—The amœbocytes are the largest cells to be found in the crustacean blood and constitute about 30 per cent of the total blood cells. Their striking appearance, due both to their very large size and to their very granular condition, has resulted in their careful study by several investigators. They vary more in form than do the corpuscles of the other groups and have been seen to undergo changes of form even while suspended in solution, away from contact with any solid body. They are true amœbocytes. The phenomena of spreading are not so extreme as in the preceding group, and when the cell processes are extended they can be withdrawn. There appears to be something of the nature of a cytotaxis existing between the corpuscles of this group, for when two cells come to lie near each other they are quite likely to move toward each other and finally to adhere.

The blood of the Isopod *Ligia oceanica* was first described by Hardy, whose observations have since been confirmed by other authors. He found three types of cells which he called eosinophil cells, basophil cells, and explosive corpuscles. The functions which these last play in the coagulation of the blood will be considered in another place.

Mollusca.—The blood cells of Anodonta include large amœbocytes, their processes being long, filamentous pseudopodia arising in large numbers on one side only of the cell. At other points of the surface of the cell large lobose pseudopodia may be formed. Other cells, nearly spherical in shape and with few processes of any sort, and large nuclei are also found.

De Bruyne recognized seven different types of corpuscles in the blood of Lamellibranchs. He used as his forms for observation Mytilus, Ostrea, Unio, and Anodonta. It seems to the author doubtful whether

the blood cells of the Lamellibranchs exhibit such differentiations as to warrant De Bruyne's classification.

In the Lamellibranch *Cardium norvegicum*, Drew was able to recognize only three types of corpuscles, all amœbocytes. These are:

1. Finely granular eosinophil corpuscles. These are the largest to be found in the blood of this form. They are supplied with large, round, or oval nuclei, have a relatively large amount of protoplasm containing numerous very small eosinophil granules, located chiefly around the nucleus. The large pseudopodia are free from granules. These corpuscles constitute about 48 per cent of the cells of the blood.

2. Coarsely granular eosinophil corpuscles. These are similar to the above, but are slightly smaller and have large granules. They constitute 44 per cent of the blood corpuscles.

3. Basophil corpuscles. These are smaller than those mentioned above and have their cytoplasm supplied with granules which stain with the basic dyes. They constitute about 8 per cent of the blood cells.

Myriapoda.—The blood of the Myriapoda is contained, for a part of its course, in definite blood vessels. There are three kinds of corpuscles found:

1. Cells having pellucid, central nuclei, invested by a few granules.

2. Oat-shaped corpuscles having very prominent nuclei.

3. Granular corpuscles in which the central nucleus is almost obscured by the large numbers of the granules. In these no definite cell wall has been observed.

Insecta.—The bloods of insects do not all contain the same histological elements. Among the different leucocytes observed in the bloods of insects may be mentioned:

1. Proleucocytes, young leucocytes with basophil protoplasm, multiplying rapidly by mitosis. From these arise the other leucocytes.

2. Phagocytes, larger than the proleucocytes, with a very hyaline protoplasm and great phagocytic powers.

3. Granular leucocytes, having their cytoplasm charged with granules of varying chromatophil reactions. These corpuscles often act as phagocytes.

4. Adipoleucocytes, cells which have the power to absorb and carry large amounts of fat.

5. Œnocytoids, large cells of a spherical form, with a homogeneous protoplasm clearly acidophil in reaction, associated with tracheæ and fat body. These cells are not phagocytic.

6. Spherule cells, cells found occasionally in the bloods of certain Coleoptera and Lepidoptera. In the latter they are especially found in the blood of the larvæ. They are leucocytes of a spherical or ovoid form, having a hyaline protoplasm charged with spherules, sometimes colorless and sometimes tinged with yellow. They are evidently related to the

so-called nephrocytes of Bruntz and the spherule cells of Kollmann found in the bloods of some invertebrates. The spherule cells of the insects do not eliminate ammonium carbonate as do the others mentioned.

The blood cells of Dytiscus and Hydrophilus appear to be of two sorts: (1) phagocytes, and (2) small round cells. The number of cells ranges from 120 to 500 per cubic millimeter in Dytiscus to 1030 to 4140 per cubic millimeter in Hydrophilus.

According to the statement of Barrett and Arnold, the blood of Dytiscus is a liquid of an amber color, turning dark brown or almost black upon exposure to the air. This fluid has a sweet odor which is slightly offensive, and evidently contains ammonia. Its specific gravity is 1.025 to 1.027. In reaction it is alkaline to litmus. The alkalinity is due to the presence of ammonium carbonate in an amount slightly in excess of that represented by a N/20 HCl solution. This blood contains about 9 per cent of total solids, does not coagulate on standing, and shows a depression of the freezing point of $-0.77°C$. It contains 6.7 per cent by volume carbon dioxide, 1.8 per cent nitrogen and no oxygen.

The blood of *Hydrophilus pissius* was found by the same observers to be a straw-colored fluid which turns dark brown upon standing in the air, but does not darken upon standing in an atmosphere of hydrogen. It has a faintly offensive odor, specific gravity of 1.012, is alkaline in reaction to litmus, its basicity being equivalent to that being represented by a N/10 HCl solution. This blood does not coagulate upon standing, contains 11.6 per cent of solids, and depresses the freezing point $-0.647°C$. Of gases it contains 3.8 per cent of carbon dioxide, 1.9 per cent nitrogen, and no oxygen. From the above statement it will appear that in the tracheate forms the blood is only doubtfully an oxygen carrier, the tissue cells receiving their oxygen through the trachael system instead of through the blood stream.

Ascidians.—In the blood of *Ascidia atra* a colorless plasma is found which is isotonic with sea water. The corpuscles are colorless and colored; the latter may be green, orange (localized in the branchial sac), and blue (in the visceral region); some are amœboid. The green cells are acid in reaction and their pigment contains vanadium in the form V_2O_5. This pigment is probably catalytic and not respiratory in character. The coagulation of the blood in this form is said to be due to an agglutination of the cells.

The blood of *Phallusia mammalata* is a colorless fluid, which upon standing first turns brown and then greenish blue. It does not coagulate and like the blood of other Ascidians is of acid reaction. The corpuscles in particular give a strongly acid reaction. The chromogen is contained only in the corpuscles. A quantitative determination showed the presence of more than 15 per cent of V_2O_5 in the chromogen.

Vertebrates.—The bloods of the vertebrates contain three general classes of cells—erythrocytes, or red blood corpuscles; leucocytes, or white blood corpuscles; and thrombocytes, or blood platelets.

Erythrocytes.—The red corpuscles or erythrocytes, as they are called, occur universally among the vertebrates. As might be expected, they are not all of the same size or form in the different groups of animals. Among all the lower vertebrates these corpuscles are oval, doubly convex, nucleated discs, containing a considerable amount of hæmoglobin. In all of the mammals, with the possible exceptions of Llama and Echidna, they are non-nucleated, and are commonly said to have the form of biconcave discs. During the past few years there has been considerable discussion as to the actual form of these cells in the circulating blood in the body. It seems now to be established that the exact form of the corpuscle is dependent to a certain degree upon the osmotic pressure of the fluid in which it is immersed for examination. In solutions having an osmotic pressure approximately equal to that of 0.9 per cent NaCl solution they show a biconcave form. In progressively less concentrated solutions (hypotonic) water is imbibed and the corpuscles swell to thin-walled cups, thick-walled cups, dimpled spheres, and finally lake-forming shadows. In hypertonic media the corpuscles shrink and become crenated. Between the limits of form induced by a 0.3 per cent NaCl solution and mild

TABLE 9.—TABLE GIVING THE DIMENSIONS, NUMBERS, AND AREAS OF ERYTHROCYTES

Animal	Diameter in microns	Number in millions per cubic millimeter	Area in square meters per liter, or square millimeters per cubic millimeter
Man	7.5	5	443.8
Chimpanzee	7.4		
Bullock	6.0	5	282.7
Horse	5.5	7	402.64
Sheep	5.0	12	471.12
Rabbit	7.0	4.8	369.4
Goat	4.1	9–10	237.6–264
Guineapig	7.2	6.3	513
Llama	4.1× 7.6	10.5–13	516–638
Chicken	12.1× 7.3	3.86	535
Duck	13.1× 7.0	2.8	432
Pigeon	12.9× 7.0	2.0	284
Lacerta agilis	16.3× 9.2	1.42	335
Eel	14.6× 8.9	1.1	224
Sole	12.0× 9.0	2.0	338
Skate	25.0×14.0	0.23	12.65
Frog	22.9×13.9	0.4–0.59	20.0
Proteus	63.5×35.1	0.036	1.26

crenation the shape of the corpuscle is repeatedly reversible. Undiluted, freshly drawn blood and blood diluted with serum of the same kind show the red corpuscles to be biconcave discs. Human serum must be diluted about one-third with water before the cup-shaped corpuscles will predominate. Freezing-point determinations, which show that the mammalian plasma is isotonic with a 0.9 per cent NaCl solution, are roughly substantiated by such dilution experiments. The collective evidence seems to justify the conclusion that the biconcave disc represents the normal shape, the concavo-convex from being an occasional modification.

The Evolution of the Red Corpuscle.—The red corpuscles found in the bloods of the higher animals may be looked upon as the results of a long-ago established habit of symbiosis. Before the bodies of the multicellular organisms had become definitely shut off from the sea, there are supposed to have entered those multicellular organisms small unicellular forms, such as amœboid or ciliated protozoa, which found in the liquid medium of the animal body a more favorable habitat than the sea water which had been their home. The sea water undoubtedly contained only small amounts of nitrogen, available for use only by these minute forms. A nitrogen hunger may be thought of as the driving force which was effective in bringing about the association. The needed nitrogen was readily obtained in these animal bodies from the products of nitrogen metabolism. In return, the unicellular form, particularly if equipped with chlorophyll, may be considered to have contributed its share by supplying carbohydrates to the other partner of the relationship, or, if not provided with chlorophyll, in some other way.

Of course, these protozoan cells cannot be looked upon as the immediate ancestors of the red blood corpuscle, but it is easy to see how they may have made such a place for themselves in the economy of the ancestral forms of the vertebrates, that the line of organisms found itself under the necessity of providing, out of its own structure, cells which could take the place of these associated cells, after the possibility of access from the sea was removed. There is good evidence pointing to the conclusion that pigment matters occurring in animal tissues were originally excreta. They are substances which are resistent to metabolism, as appears from the fact that they are stored in the more aged cells, and some of them are substances which diffuse but slowly, if at all, through animal membranes. Such substances, nitrogenous in character, would be seized by such symbiotic cells as have been hypothesized. These cells, filled with their burden of pigment, would then be free-wandering pigment cells. If, then, the pigment happened to be some substance showing a remarkable affinity for oxygen, there would be provided for the organism a mechanism for the transport of oxygen which could not help but be of great service.

There are no cells among the invertebrates which are exactly comparable with the erythrocytes of the vertebrates. Instances do occur, as, for example, in Solen, Glycera, Capitella, and Phoronis, in which nucleated, amœboid cells possess inclusions of pigment matter, but so different are these cells in appearance from ordinary erythrocytes as not to be confused with them. In the very great majority of cases such blood pigments as exist among the invertebrates are to be found in solution.

The utilization of any pigment for the transport of oxygen appears to be a rather haphazard matter among the invertebrates. Here and there are scattered species of animals which make use of one or another such substances, but the distribution of these substances among different forms is such as to seem to indicate that these particular lines have really very far to go before they become really settled in habit. Closely related forms make use of widely differing substances. Also the means employed for the conservation of the substance for respiratory purposes fall far short of the methods employed by the vertebrates. In the lowest forms of animals in which hæmoglobin and hæmocyanin appear they are in solution in a plasma. So far as the author is aware, there is no elaborated scheme provided for the preservation of hæmocyanin, unless it be among the Cephalopods. Loss of hæmoglobin is, however, prevented by having the fluid in which it is dissolved kept within the walls of a closed system of tubes. Such systems become quite complete and efficient in some forms of worms and crustaceans, becoming very finely divided so as to reach to the farthest limits of the body. Such systems of tubes are, however, subject to injury, and if a tube is broken there is the possibility that a large part or the whole of the oxygen-transporting pigment may be lost.

One of the substances likely to be ingested by such symbiotic cells as has been suggested would be hæmoglobin or some ancestral form of it then present, whereupon there would be formed the prototype of the modern erythrocyte. In the lowest forms in which hæmoglobin occurs it is always in solution. Its incorporation into corpuscles occurs in the higher forms. It seems logical to suppose that phylogenetically it appeared long before it became included within corpuscles. It has rather close relations, chemically, with the chlorocruroins, echinochromes, histohæmatins, hæmocyanin, myohæmatin, neurohæmatin, and hæmerythrin. These pigments are widely distributed among organisms and also in a very haphazard manner among the tissues of the forms in which they occur. The reasons which underlie the distribution are certainly not clear. The pigments may be found in the vascular fluids, the muscular tissues, nervous tissues—anywhere. Such forms of animals as make much use of their muscles may have large amounts of myohæmatin—but this is not of necessity the case. The pigments seem likely to be produced as waste end products in some special line of metabolic activity. The organisms which produce them may excrete them from the

body, though they are more likely to segregate them into the cells of the skin, or they may utilize them for special purposes.

When the usefulness of the pigments to the organisms had once been established, it would seem to be a short step to arrive at a condition in which the processes leading to the production of the pigment would be emphasized for the sake of the waste end product rather than for what had been the original purpose of the process.

The lack of the red corpuscles in the lower forms may be correlated with the condition existing in the developing embryos of higher animals. The first fluid to circulate in the heart and blood vessels of an organism is one devoid of corpuscles. At an early date the lymphocytes appear, and after them the erythrocytes. The erythrocytes formed in the bone marrow of the higher animals contain not only hæmoglobin, but nuclei. The nuclei soon disappear and with their disappearance there is probably a considerable diminution in the individual metabolism of the corpuscles.

Leucocytes.—A second great group of the cells found in the bloods of the higher forms, such as mammals, are the leucocytes, or white blood corpuscles, as they are more familiarly called. As a matter of fact, the functions of all of these cells are not clearly understood. They are certainly not all of the same type, from a morphological point of view. Numerous classifications have been elaborated by different authors in the hope of giving a clearer understanding of the place occupied by these different cells in the economy of the organism. None of these classifications has proved thoroughly acceptable, though that of Ehrlich has probably been most widely approved. According to Ehrlich, white blood cells may be divided into two main groups, lymphocytes and leucocytes.

1. *Lymphocytes.*—These are colorless cells, non-granular in appearance, and capable of changing form, though probably not for locomotor purposes. Of these there are two groups, small and large.

a. Small lymphocytes are cells having about the size of erythrocytes, in which the cytoplasm is reduced to a very thin film about the rather large and homogeneously staining nucleus. These comprise from 20 to 25 per cent of all the white corpuscles.

b. Large lymphocytes are two or three times as large as the preceding, with nucleus somewhat eccentric and cytoplasm more abundant than in the smaller corpuscles and non-granular. They comprise less than 1 per cent of the white corpuscles.

2. *Leucocytes.*—The leucocytes are cells characteristically amœboid, with granules of different sorts in the cytoplasm.

a. Transition forms (uninuclear leucocytes) have a single large nucleus, more or less lobulated, and cytoplasm abundant and fairly granulated. The granules stain with neutral stains and are therefore referred to as neutrophil granules. The name *transition form* implies that these

leucocytes represent an intermediate stage between the large lymphocytes and the following variety, though this contention is vigorously opposed by many competent hæmotologists.

b. Polymorphonuclear Leucocytes.—The nucleus is segmented into lobes connected by narrow strands. The cytoplasm is amœboid and is granular. The typical cells of this sort form about 60 to 75 per cent of the white corpuscles of the blood. The eosinophil cells form a subdivision of this group. They have a similarly segmented nucleus, but the cytoplasm contains numerous coarse granules that stain with acid dyes, such as eosin, whence the name. In the case of the fish the eosinophil cells have received some special attention. Many observers have failed to discover in the blood of the fish any eosinophil cells. Drury finds that this supposed absence of the coarsely granular cells is not real but is in part due to the fact that the granules of the cells are peculiarly unstable. This instability evidences itself by the melting and fusing of the granules and also by changes in their staining properties. These cells are intimately connected with the ingestion of bacilli and are very susceptible to the action of their extracts, the presence of these causing disintegration of the cells. Such an action is comparable to the actions observed in the case of the coarsely granular eosinophil cells of all the animals which have been examined. It seems, therefore, justifiable to consider that the function of the unstable cells is that of the stable cells. Others have considered these to be cells excreting their granules and so forming toxins for the destruction of bacteria.

c. Mast Cells.—These cells exist in very small numbers, under normal conditions comprising less than 1 per cent of the corpuscles of the blood. They have a polymorphic nucleus like the corpuscles of the preceding group, but differ in the fact that the granules in the cytoplasm are strongly basophilic, *i.e.*, stain only with basic dyes.

3. *Thrombocytes, or Blood Platelets.*—A third class of blood cells found in the bloods of vertebrates is the thrombocyte. The thrombocytes are the smallest of the blood cells, and though much study has been given them there is still some doubt as to their true nature. In the first place, it seems very probable that the small circular discs, of a very glistening appearance, seen in freshly spread blood, are simply the nuclei of these minute blood cells. The protoplasm is very unsubstantial and apparently breaks up almost immediately when the blood is shed from the blood vessels. The corpuscles seem to be of the same general sort as the explosive corpuscles described above as forming an important constituent of crustacean blood. In this case, however, it would seem that the corpuscles are even more sensitive than are the crustacean explosive corpuscles. It is a matter of great difficulty to prepare thrombocytes in such a way as to exhibit the cytoplasm at all, to say nothing of observing in them the occasionally described amœboid movements which are said to be charac-

teristic of these cells. The part which these cells may play in the coagulation of the blood will be touched upon in another place.

The view of the blood platelets just suggested is by no means the only one held by physiologists. Marchesini contends that there are three classes of erythrocytes—stable, partly stable, and unstable. It is the disintegration of the unstable leucocytes which leads to the formation of blood platelets out of the débris. The leucocytes serve merely as centers about which gathers the débris of the unstable erythrocytes. He claims that the process of the formation of the platelets can be watched most instructively in the blood of frogs and fowls.

Fry also contends that the blood platelets in man arise from the erythrocytes. It is a matter of interest that not all bloods contain these elements; for example, the blood of fishes is devoid of them.

CHAPTER X

THE BLOOD AS AN OXYGEN CARRIER

As pointed out in the preceding chapter, the organism has need of a circulating fluid in order properly to supply food materials to the tissues and to carry away from the tissues waste materials destined to be eliminated from the body. The function of the blood and other body fluids as carriers of oxygen requires further discussion. The body fluids of all the higher organisms, with the possible exception of the insects, cannot be considered as merely water containing food matters in solution. There is in the blood of all the higher forms a protein, usually pigmented, which carries oxygen from one part of the body to another. The need of such a carrier is evident when the comparatively limited oxygen-absorbing power of water is considered, and this is compared with the demands made by the cells of the animal body for oxygen and the amount of liquid in the body which can be given up to oxygen transport. The fact that the substances used as oxygen carriers are proteins is, in itself, very significant. Proteins are substances which dialyze slowly, or not at all, through the membranes of the cells composing the animal body. Hence they are substances which can be conserved readily. If the body were to make use of carriers which could readily dialyze, there would be the necessity of continuous reconstruction of a very valuable material. The proteins when once built can be utilized over and over again.

Solubility of Oxygen in Water.—Water has the power to absorb oxygen, in accordance with the well-known gas laws, in definite though comparatively small amounts. The following table gives an indication of the proportions in which the various gases of the atmosphere are dissolved in certain waters. The figures in each case represent the number of cubic centimeters of gas per liter at 760-millimeter atmospheric pressure.

TABLE 10.—OXYGEN ABSORPTION. (*From Birge and Juday*)

Number of cubic centimeters of oxygen absorbed by 1 liter of distilled water at different temperatures from a free dry atmosphere of 760-millimeter pressure

Temperature	0	1	2	3	4	5	6	7	8	9
−2	10.880	10.850	10.820	10.790	10.760	10.730	10.700	10.670	10.640	10.610
−1	10.580	10.551	10.522	10.493	10.464	10.435	10.406	10.377	10.348	10.319
0	10.290	10.263	10.236	10.209	10.182	10.155	10.128	10.101	10.074	10.047
1	10.020	9.993	9.966	9.939	9.912	9.885	9.858	9.831	9.804	9.777
2	9.750	9.725	9.700	9.675	9.650	9.625	9.600	9.575	9.550	9.525
3	9.500	9.476	9.452	9.428	9.404	9.380	9.356	9.332	9.308	9.284
4	9.260	9.237	9.214	9.191	9.168	9.145	9.122	9.099	9.076	9.053
5	9.030	9.008	8.986	8.964	8.942	8.920	8.898	8.876	8.854	8.832
6	8.810	8.789	8.768	8.747	8.726	8.705	8.684	8.663	8.642	8.621
7	8.600	8.580	8.560	8.540	8.520	8.500	8.480	8.460	8.440	8.420
8	8.400	8.381	8.362	8.343	8.324	8.305	8.286	8.267	8.248	8.229
9	8.210	8.191	8.172	8.153	8.134	8.115	8.096	8.077	8.058	8.039
10	8.020	8.002	7.984	7.966	7.948	7.930	7.912	7.894	7.876	7.859
11	7.840	7.824	7.808	7.792	7.776	7.760	7.744	7.728	7.712	7.696
12	7.680	7.664	7.648	7.632	7.616	7.600	7.584	7.568	7.542	7.526
13	7.520	7.505	7.490	7.475	7.460	7.445	7.430	7.415	7.400	7.385
14	7.370	7.355	7.340	7.325	7.310	7.295	7.280	7.265	7.250	7.235
15	7.220	7.206	7.192	7.178	7.164	7.150	7.136	7.122	7.108	7.094
16	7.080	7.066	7.052	7.038	7.024	7.010	6.996	6.982	6.968	6.954
17	6.940	6.927	6.914	6.901	6.888	6.875	6.862	6.849	6.836	6.823
18	6.810	6.798	6.786	6.774	6.762	6.750	6.738	6.726	6.714	6.702
19	6.690	6.678	6.666	6.654	6.642	6.630	6.618	6.606	6.594	6.582
20	6.570	6.559	6.548	6.537	6.526	6.515	6.504	6.493	6.482	6.471
21	6.460	6.449	6.438	6.427	6.416	6.405	6.394	6.383	6.372	6.361
22	6.350	6.339	6.328	6.317	6.306	6.295	6.284	6.273	6.262	6.251
23	6.240	6.230	6.220	6.210	6.200	6.190	6.180	6.170	6.160	6.150
24	6.140	6.130	6.120	6.110	6.100	6.090	6.080	6.070	6.060	6.050
25	6.040	6.030	6.020	6.010	6.000	5.990	5.980	5.970	5.960	5.950
26	5.940	5.930	5.920	5.910	5.900	5.890	5.880	5.870	5.860	5.850
27	5.840	5.831	5.822	5.813	5.804	5.795	5.786	5.777	5.768	5.759
28	5.750	5.741	5.732	5.723	5.714	5.705	5.696	5.687	5.678	5.669
29	5.660	5.651	5.542	5.633	5.624	6.615	5.606	5.597	5.588	5.579
30	5.570	5.561	5.552	5.543	5.534	5.525	5.516	5.507	5.498	5.489

Respiratory or Chromoproteins. It is evident that if an animal is to make use of very much oxygen it will be obliged to have a very large

absorbing surface and a very large amount of transporting fluid unless some other carrier than water can be found, the water in this case being represented by the plasma of the blood or other body fluid. In various animal groups certain complex substances which unite readily with oxygen have been elaborated. These substances combine with oxygen at ordinary atmospheric pressure and dissociate at somewhat reduced pressures, so that the oxygen which had been bound can readily escape into the plasma. Such proteins are the following:

TABLE 11

Protein	Color	Carbon	Hydrogen	Nitrogen	Iron	Sulphur	Oxygen	
Hæmoglobin	Red	636	1025	164	1	3	189	
Hæmoglobin	Red	600	960	154	1	3	179	
Hæmocyanin	Blue	857	1363	222	Cu	4	258	
Chlorocruorin	Green	560	845	143	Fe	3	167	
Hæmerythrin	Red	427	761	135	Fe	2	153	
a-Achroglobin	Colorless	523	761	196	..	1	140	Patella
b-Achroglobin	Colorless	621	814	175	..	1	169	Chiton
g-Achroglobin	Colorless	721	915	194	..	1	183	
d-Achroglobin	Colorless	659	792	165	..	1	153	
Pinnaglobin	Colorless	720	985	183	Mn	4	210	
Hæmatoporphyrin	(?)	68	74	8	..	..	12	or
		34	36	4	..	..	6	or
		16	18	2	..	..	3	coming from
Hæmatin	.....	32	36	4	Fe	..	3 plus $3H_2O$	

A blue pigment, hæmosycotypin, found in some of the molluscs, has essentially the composition of the hæmocyanin, but contains in the molecule one atom each of copper and zinc. Hæmatoporphyrin is doubtfully considered a respiratory pigment.

Chlorocruorin.—Blood containing a green pigment was found by Milne-Edwards in 1838 in one of the Sabella-like forms of the annelid worms. Shortly afterward Dujardin and Quatrefages made similar observations on other annelids. Lankester found the green pigment chlorocruorin in the blood of the annelid Siphostoma, and Krukenberg secured green blood when he cut off the gilled filaments from Spirographis and collected the liquid which escaped.

The spectral properties of this pigment have been studied by Lankaster and by Griffeths. The pigment exists in two forms, one oxidized and the other reduced. Those interested may find a description of the spectrum in von Furth's work on comparative physiology of the invertebrates. Lankaster bases his claim that chlorocruorin is a respiratory pigment upon the fact that when a solution of the pigment in the reduced form is shaken with air it assumes the oxidized form. While Krukenberg expressed himself as not being convinced as to the respiratory value of the pigment, the work of Griffeths appears to establish its position.

As will be noticed from an examination of the formulas as given in the table, this substance presents a molecular constitution very similar to that of hæmoglobin. One can hardly fail to be impressed with the similarity, and feel some degree of certainty that the functions of the two substances are similar. Both pigments give a blue color with guaiac tincture.

It is a matter of some interest that Milne-Edwards found that some of the very closely related forms, such as Serpula, Terebella, and Hermella, have red blood instead of green. Similar differences in the blood pigments of rather closely related forms will be found in other groups of animals.

Hæmoglobin: Its Physicochemical Character.—Hæmoglobin is a compound of a globin (a simple protein allied to the histones) with a crystalline substance, hæmatin, of the composition $C_{32}H_{32}N_4FeO_4$. It is remarkable for the comparative ease with which it crystallizes out of solution, it being one of only a few protein bodies which have to this time been crystallized. Attention should be directed to the significance of the iron atom in the hæmatin molecule, for it is to this, probably, that the oxygen is linked in the formation of oxyhæmoglobin.

Hæmoglobin has the property of combining readily with certain gases with which it may happen to come into contact, forming with them readily dissociable compounds (except with carbon monoxide). On account of this behavior a variety of forms of hæmoglobin has been described, such as oxyhæmoglobin, reduced hæmoglobin, carbon monoxide hæmoglobin, carbohæmoglobin, and methæmoglobin. These substances must be considered as definite, though temporary, chemical compounds rather than simple adsorptions of gas upon the hæmoglobin molecule. The different hæmoglobin compounds may be recognized in a variety of ways.

The substance hæmoglobin is widely distributed throughout the animal kingdom, as will be pointed out more fully in another connection, and shows rather wide ranges of chemical composition. That the hæmoglobins are actually different may be shown in a variety of ways, as, for example, by spectroscopic examination. As sunlight passes through a solution of hæmoglobin, some of the light rays are absorbed by the solution, leaving dark bands across the spectrum. The position and the breadth of the bands differ in the different hæmoglobins and in different concentrations of the same hæmoglobin.

The hæmoglobin taken from the arteries near the heart is for the most part oxyhæmoglobin; that taken from the veins is a mixture of oxyhæmoglobin and reduced hæmoglobin. A comparison of the spectra of these two forms shows decided differences in light absorption. In the same way solutions of carbon monoxide hæmoglobin and methæmoglobin show very different arrangements of the absorption bands. The colored chart

(Fig. 11) shows the spectral appearances of the different forms of hæmoglobin mentioned as well as some other pigment forms. It will be noted that, while the oxyhæmoglobin presents two absorption bands in the yellow and the green of the spectrum, the reduced hæmoglobin has only one broad band covering the yellow-green portion of the spectrum. Blood which has been standing in the open air for some time gradually changes in color. This is due to a change in the hæmoglobin to the methæmoglobin form. Such a change can also be brought about by the use of certain chemicals, such as potassium ferrocyanide or potassium permanganate. This compound appears to contain about the same amount of

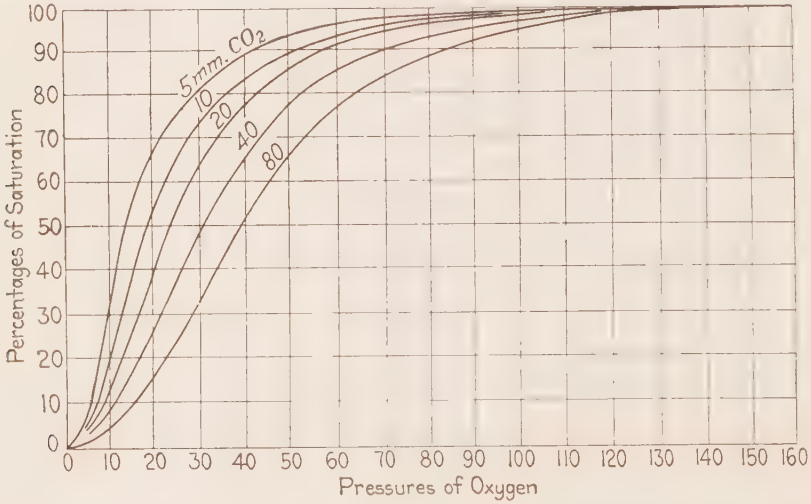


FIG. 12.—Curves of dissociation of the oxyhæmoglobin at different pressures of oxygen. Five curves are shown to indicate that the dissociation of the oxyhæmoglobin is greatly influenced by the presence of CO_2. The figures along the ordinates (10 to 100) indicate percentages of saturation of the hæmoglobin with oxygen, while the figures along the abscissa (0 to 160) indicate different pressures of oxygen. The curve marked "5 mm. CO_2" shows the amount of combination of oxygen and hæmoglobin when the CO_2 is absent or present only in traces. In this curve at a pressure of 30 millimeters of oxygen it will be seen that the hæmoglobin is 80 per cent saturated with oxygen, while with a pressure of 40 millimeters of CO_2, which approximates that in the body, the hæmoglobin at the same pressure of oxygen is only 50 per cent saturated. (*From Howell, after Bohr.*)

oxygen as the oxyhæmoglobin, but it is combined into such a stable form that this hæmoglobin will not serve as a respiratory protein. If methæmoglobin be treated with a reducing solution, such as ammonium sulphide, this stable compound is transformed into reduced hæmoglobin.

Dissociation of Oxyhæmoglobin.—The value of hæmoglobin as an oxygen carrier is associated with its power to combine chemically with oxygen at the oxygen pressures found in atmospheric air or even in the air sacs of the lung, and to dissociate, thus giving off oxygen at lower oxygen pressures such as are found in the body tissues and cells. As the result of many investigations, having apparently discordant results, it

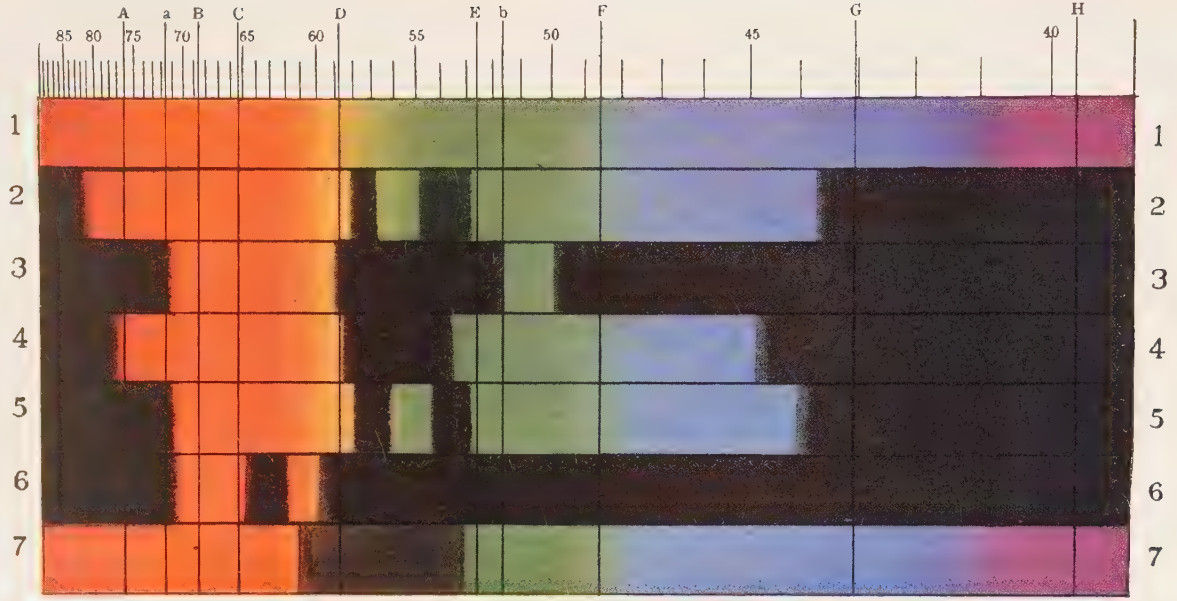


FIG. 11.—Solar spectrum and absorption spectra of blood pigments. Scale numbers give wave lengths in one hundred-thousandths of a millimeter.

1. Solar spectrum showing principal Fraunhofer lines.
2. Spectrum showing absorption bands caused by passing the light through either a very dilute solution, or through a very thin layer of solution of oxyhaemoglobin.
3. Spectrum of oxyhaemoglobin, more concentrated.
4. Spectrum of reduced haemoglobin.
5. Spectrum of carbonmonoxide haemoglobin.
6. Spectrum of methaemoglobin.
7. Spectrum of haemocyanin.

(*Facing page* 128)

appears that the combining power of the hæmoglobin for oxygen is conditioned by a number of variable factors. Among these are the pressure or tension of the gas, the temperature, the salt content of the blood, and especially the carbon dioxide content (which may mean the hydrogen-ion concentration arising from the dissociation of carbonic acid) of the blood. The effect of the presence of the carbon dioxide is, at all pressures, except the extremes, to lower the point at which the dissociation of oxyhæmoglobin into oxygen and hæmoglobin takes place. Figure 12 shows graphically the extent to which the presence of carbon dioxide affects the hæmoglobin-oxygen combination. The dissociation curve for human

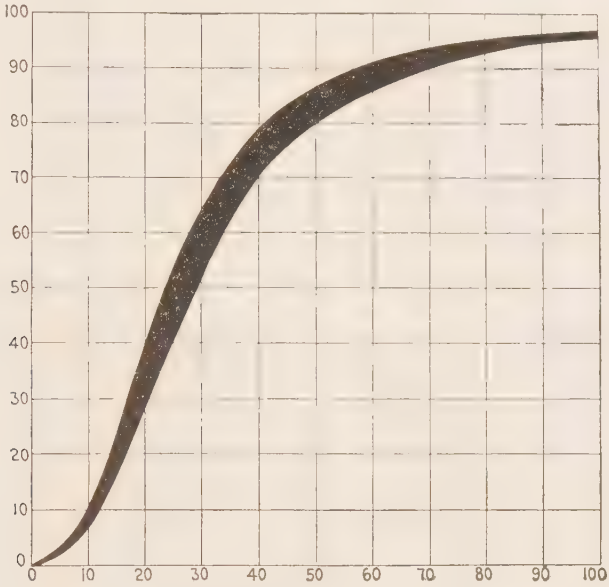


FIG. 13.—The dissociation curve for human blood. The thickness of the curve indicates the limits within which the curve may fall for normal persons. The figures along the abscissa indicate the pressure of oxygen in millimeters of mercury; those along the ordinate indicate the percentage saturation of the hæmoglobin with oxygen. (*From Howell, after Barcroft.*)

blood showing the limits of the variations which may naturally be expected in the oxyhæmoglobin dissociation because of individual differences in blood composition is shown in Fig. 13.

The Transport of the Hæmoglobin in the Body.—Different animals vary in the manner in which they transport the blood pigment to the different regions of the body. In the earthworm and similar forms and in some of the Crustacea the hæmoglobin is carried in solution in a liquid plasma contained in a set of closed vessels of the so-called pseudohæmal system. In the higher animals the hæmoglobin forms the larger portion of the content of certain special cells or corpuscles which are carried in a colorless plasma within the closed system of vessels of the vascular

system. In some intermediate forms the hæmoglobin is collected in the form of small drops within the cytoplasm of more or less amœboid cells. The change in the habit of hæmoglobin carrying in the higher animals is doubtless an adaptation favoring the conservation of the substance which might be the more readily lost from a solution than when contained within some more or less firm envelope.

The Amount of Hæmoglobin in the Blood.—The percentage of hæmoglobin in the bloods of different animals is subject to great variation. In the lower forms the amount is relatively smaller than in the higher forms. In the human blood there are approximately 14 grams of hæmoglobin for

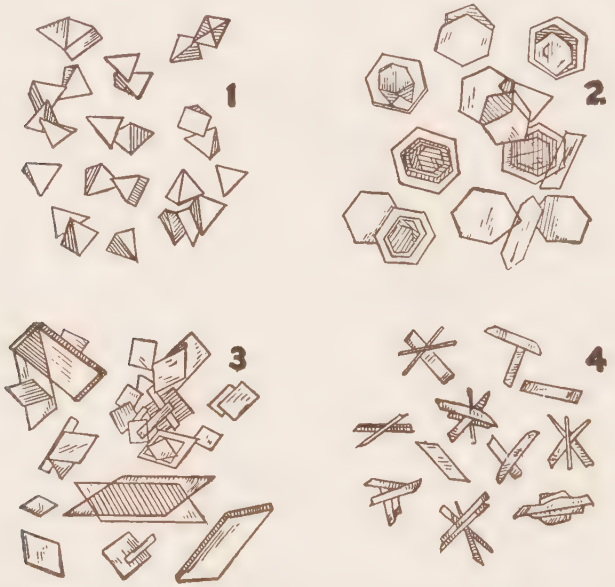


FIG. 14.—Sketches to indicate the forms of hæmoglobin crystals as obtained from blood of different species. 1, guineapig; 2, squirrel; 3, horse; 4, rat. (*From Mitchell, "General Physiology," after Reichert. By permission of the McGraw-Hill Book Company, Inc.*)

every 100 cubic centimeters of blood. In the human body the blood comprises about 5.0 per cent of the total weight. This means, in the case of a man of 150 pounds (68 kilograms), that there are about 7.5 pounds, or 3.4 kilograms, of blood, amounting to about 3.213 liters. In this amount of blood there are about 450 grams of hæmoglobin. If the blood contains 5,000,000 corpuscles per cubic millimeter there will be about 16.065 trillion corpuscles carrying this 450 grams of hæmoglobin, and these corpuscles will have a surface of some 1700 square meters. Each corpuscle would then carry about 3×10^{-11} grams of hæmoglobin. One gram of hæmoglobin has the power to combine with about 1.36 cubic centimeters, or 0.0019 gram, oxygen at 760-millimeter pressure. The whole blood supply would, therefore, be able to combine with 611.7

cubic centimeters, or .855 grams. If one molecule of oxygen combines with one molecule of hæmoglobin a rough calculation of the combining weight of hæmoglobin may be made from the proportion

1 gram Hb:0.0019 gram::x:32, in which case x = 16,842.

Recent investigations suggest that hæmoglobin actually has a molecular weight nearly four times this value.

Hæmoglobin Crystals.—Hæmoglobin is one of only a few proteins which it has been found possible to obtain in crystalline form. Reichert and Brown have made a very extended study of the forms of crystallization of hæmoglobins from different animals. Between the crystals of many of these forms there is a distinct similarity. Other hæmoglobins are, however, quite widely variant from the more general type, some being so unlike as to raise a question concerning the identity of the substance. The question thus arises, Is hæmoglobin a definite substance, or are there many different hæmoglobins? The variations observed in the crystallization of the hæmoglobins may be due to the differing salt contents of the different bloods (see Fig. 14). It is also true that there may be differences in the globin part of the molecule. It would be difficult to imagine that the globins of the earthworm, of the mollusc Planorbis, of fishes, amphibians, reptiles, birds, and mammals would be identical in composition and structure. There is certainly evidence that corresponding proteins of different animal groups present differences of structure and composition. Such differences of structure and composition probably occur in the hæmoglobin as well as in other protein molecules, and if they do occur they would account for some of the differences in crystallization which have been observed.

Fig. 15.—Hemin crystals. (*From Mitchell, "General Physiology." By permission of the McGraw-Hill Book Company, Inc.*)

The hæmatin portion of the hæmoglobin molecule appears to be more constant. Hæmin crystals (Fig. 15) from the bloods of the earthworm, frog, and mammal are so closely alike as safely to be regarded as identical. Of course, this portion of the molecule is less complex than the protein portion and is therefore subject to less possibility of modification.

Hæmoglobin among the Worms.—Among the worms hæmoglobin occurs very generally in the blood and fluids of the body cavity. In the blood it is usually dissolved in the plasma and does not often appear in the corpuscles, as it does among the vertebrates. There are a few cases in which the cellular elements of the blood contain hæmoglobin, as in Glycera, Capitella, Phoronis, and others. The records of the red blood of some of these forms date back to the work of Swammerdam, about the middle of the seventeenth century. It is to be noted, however

that whenever hæmoglobin occurs in cells these are not like the red blood cells of the vertebrates, but more resemble white corpuscles, each cell containing a small mass of the pigment in the cytoplasm.

The presence of iron in the ash of the red blood of certain segmented worms was noted by Hunefeld in 1839, and Quatrefages noticed that the red color of the blood was independent of the cellular elements. Rollet showed by means of Teichman's test (hæmin crystals) that the red color of the blood of worms was due to the presence of hæmoglobin, and this contention was made sure by the spectroscopic work of Nawrocki and of Preyer. In England, Griffeths took the red blood from some 500 earthworms and made a careful chemical analysis of the red coloring matter and obtained results which are closely like those which have been obtained from similar analysis of the hæmoglobin of vertebrates. Table 12 shows the results of the various analysis upon worm blood and upon vertebrate blood.

TABLE 12

	Earthworm	Average of different vertebrates
Carbon	53.86–53.91	51.15–54.87
Hydrogen	7.02– 7.10	6.76– 7.39
Nitrogen		16.17–17.94
Sulphur	0.37– 0.41	0.39– 0.85
Iron	0.39– 0	0.33– 0.59

One author has been found who claims that the red color of the blood of Lumbricus is due to the presence of hæmatoporphyrin. This pigment is undoubtedly found in the tissues of the body, but according to the best information is not found in the blood. The chemical composition of this substance would not inspire much confidence in it as a carrier of oxygen.

In Arenicola, Barcroft and Barcroft have shown that the hæmoglobin dissolved in the vascular liquid is a different substance from the hæmoglobin found in human blood. The spectral bands are somewhat displaced from the usual positions, the *a*-oxyhæmoglobin band being 18 Ångstrom units and the *a*-carboxyl band being 11 Angstrom units nearer the violet than in the human blood.

This hæmoglobin has a much greater affinity for oxygen and much less affinity for carbon monoxide than human hæmoglobin. This greater affinity for oxygen makes it possible for the gas exchanges to take place at much lower oxygen pressures than is customary for human blood, pressures of from 1 to 3 millimeters of mercury of oxygen being sufficient. Carbon monoxide, instead of having 250 times the affinity for oxygen, has only 70 to 50 times the affinity for oxygen for the hæmoglobin of Are-

nicola. The oxygen capacity of the blood of Arenicola is about 0.01 cubic centimeter of oxygen per gram of worm.

Hæmoglobin among the Echinodermata.—Hæmoglobin is found also in the mud-dwelling Thyone, a Holothurian. Here it is found in small nucleated corpuscles which might easily be mistaken for the red corpuscles of the higher animals except for the fact that they are nucleated, and are somewhat larger than the mammalian blood cells. The presence of hæmoglobin here would seem to be correlated with the habit of the animal, living as it does under conditions rather unfavorable for a good oxygen supply.

Hæmoglobin among Mollusca.—While hæmoglobin, as has been seen, is very widespread in its distribution among the worms, it is to be found only in scattered cases among the molluscs. Up to this time it appears to have been described in such forms as the pulmonate Planorbis and in some of the mussels.

In Planorbis the pigment is dissolved in the plasma of the blood and is not found in the blood corpuscles. The identification of this pigment as a hæmoglobin was the result of the work of a number of investigators. The iron content of the blood of Planorbis was noted; it was shown that the blood turns blue when treated with guaiac solution—a recognized test for hæmoglobin; the position of the absorption bands was found practically identical with that of other known hæmoglobin. All of these facts lend emphasis to the view that the pigment in the case of Planorbis is not essentially different from the hæmoglobin found in higher forms.

While in Planorbis the hæmoglobin is dissolved in the blood plasma, in the mussels it is found within the blood cells. According to the account given by Cuenot, if the shell of a specimen of Arca is opened there is seen in the mantle a considerable collection of red blood which comes from the contraction of a sinus in the foot. Upon puncture, the blood will flow freely and may be collected in a tube. Upon standing, the blood cells fall to the bottom of the tube and the liquid above them remains colorless. The red blood cells are ovoid, lenticular bodies, which are much more numerous than the colorless cells of the same blood.

At oxygen tensions down to 7 per cent (one-third that present in the air at normal barometric pressure), Planorbis never makes use of its hæmoglobin, which remains saturated; nor does it make use of its lung, since diffusion through the surfaces exposed to the water is sufficient to supply its needs. Below 7 per cent tension the oxygen in physical solution in the blood no longer suffices, the hæmoglobin is reduced, and the lung is frequently filled with air. Down to an oxygen tension of 7 millimeters, or about 1 per cent, Chironomous larvæ do not use their hæmoglobin, but from about 6 millimeters down they do. The hæmoglobin dissociation curves for the two animals are different. The results lead to

the conclusion that in these two forms the hæmoglobin, by its power of binding oxygen, chemically renders available sufficient oxygen for the needs of the animals at oxygen tensions so low that the necessary amount is not supplied by simple solution. The hæmoglobin does not act as a store for oxygen but is alternately oxidized and reduced as the blood circulates between the lung and the tissues in Planorbis, or between the surface of the body and the interior of Chironomous. In each case the hæmoglobin is present in just such a solution as to enable it to utilize those low tensions of oxygen to which it is liable to be subjected.

The amount of oxygen present in the blood of such animals as these under discussion is not large, the oxygen capacity of Planorbis blood being estimated at from 1.2 to 1.5 volumes per cent and in the case of Chironomous 6 volumes per cent. The quantity of blood in Planorbis is about one-third the body weight, and in Chironomous about one-half the body weight. It has also been shown that Planorbis uses 1.09 milligrams of oxygen per gram of body weight per minute, and Chironomous 2.675 milligrams of oxygen per gram of body weight per minute.

Lankester, in 1871, described the appearance of hæmoglobin in the blood of certain of the Crustacea, such as Daphnia and Cheirocephalus. While in the higher forms of the Crustacea the oxygen-carrying function is performed by hæmocyanin, and hæmoglobin is not to be found, the latter pigment is found quite generally in the bloods of the lower members of the phylum. It occurs in certain of the phyllopods like Daphnia, in Apus (according to Lankester), in Branchipus (Regnard and Blanchard), and finally in the bloods of some of the Copepods, Lernanthropus, Clavalla, and Congericola.

In these last-named crustaceans Van Beneden discovered that there is a double circulation similar to that found in the Annelida. There is, on the one hand, a lacunar system with a colorless fluid containing numerous, uncolored corpuscles, and on the other hand a complex vascular system of definite closed tubes, in which there circulates a red, hæmoglobin-containing fluid which contains few if any cells. There is no communication between the two sorts of circulating fluids. Here, as in annelids, it must be considered that the red blood fulfils the functions of the red corpuscles of the higher animals. The red blood does not appear to be a food carrier in any sense of the term, as it does not diffuse to any great degree through the walls of the blood vessels. In both the Annelida and Crustacea it appears that the red fluid furnishes oxygen to the cœlomic fluid, which, in turn, passes it directly to the active cells of the body. The pigmented fluid may also serve in the carrying of carbon dioxide, though this has not as yet been clearly established.

The blood of the Branchiopods is at certain periods intensely red in color. In Artemia and Branchipus the young individuals have been found to contain red blood while the blood of the adults is uncolored.

In these cases the color appears to be limited to the plasma and not to pigment held in the corpuscles.

The Significance of Hæmoglobin in Lower Forms.—The physiological significance of the appearance of hæmoglobin instead of hæmocyanin in these forms is not entirely clear. The suggestion of Cuenot that Planorbis has a supply of hæmoglobin so as best to make use of the scanty oxygen supply of stagnant pools may be significant. What is known concerning the relative oxygen-carrying capacity of red as compared with blue blood certainly adds weight to such a suggestion. It is worthy of note that in certain of the Gastropods in which the pigment is lacking in the blood, it does appear in the muscles of the pharynx. This is true in Lymneus and Paludina. Cuenot also says that hæmatin appears in the snails in the form of a bile pigment stored up in the liver, and that it can be made serviceable under certain conditions in the form of hæmoglobin for respiration. In very closely related forms only white blood is found, as in Arca noe, Arca barbata, and Solenensis. Cuenot showed that the forms which contain hæmoglobin have the habit of living very closely together, in some cases so closely as not to be able to open their shells. The renewal of the water for breathing purposes takes place, therefore, with considerable difficulty. It would be an interesting experiment to bring the young of the hæmoglobin-carrying species into positions where they will have plenty of room, and where copper and not iron will be present in the surrounding water.

An attempt has recently been made by Bürker to establish a definite correlation between the area of the red blood corpuscle and its hæmoglobin content. This attempt has been most carefully reviewed by Ponder, with the result that it seems safe to say that there is not sufficient evidence at the present time upon which to base a statement that such a correlation exists. The correlation may exist, but the present methods of measurement of the sizes of the corpuscles are so crude that it is unwise to base such a far-reaching conclusion upon the measurements made up to this time.

The Rôle of Hæmoglobin as a Carbon Dioxide Carrier.—It has been commonly stated that the hæmoglobin serves as the carrier of oxygen, and that when the oxygen is given off, the hæmoglobin exists in the reduced form. From recent investigations by Henderson, Van Slyke, and others it appears that the carbon-dioxide-carrying function of the hæmoglobin is equally important. The question as to whether the carbon dioxide simply replaces the oxygen in the hæmoglobin complex is one yet to be settled, though the present evidence indicates that in a combination between hæmoglobin and carbon dioxide the carbon dioxide becomes attached to a different portion of the molecule from that to which the oxygen is normally attached. Inasmuch as the presence of hæmoglobin in the blood makes it possible for the blood to carry an increased amount

of carbon dioxide without changing the pH of the blood more than the normal difference between arterial and venous blood, it may be said that the hæmoglobin acts as a buffer substance. (For a brief discussion of buffer actions as related to carbon dioxide transport see a later section.)

Hæmocyanin.—The bloods of very many of the Mollusca and Crustacea are characterized by their bluish color. This is due to the presence of an interesting copper-containing pigment, hæmocyanin. From the time of Erman, who made a note in 1816 of the blue color of blood of some of the Mollusca, there have been frequent mentions in the literature of similar observations. Our knowledge as to the nature and function of this substance has been very gradually accumulated, and is still far from complete.

Distribution of Hæmocyanin.—According to von Furth, hæmocyanin exists in the blood of the following Molluscs:

Lamellibranchiata	Gastropoda	Cephalopoda
Mytilus	Helix	Octopus
Anodonta	Limnæus	Sepia
Unio	Arion	Eledone
Mya	Fissurella	Loligo
Pecten	Haliotis	
	Turbo	
	Murex	
	Cassidaria	
	Triton	
	Cyclostoma	
	Scaphander	

Hæmocyanin is the respiratory protein commonly found in the bloods of Crustacea. Great variations, however, occur in the amount of the substance found in different genera, and sometimes the amount has been so small as to be overlooked. In other cases a pigment of a more prominent color is present in such amount as to obscure the presence of the hæmocyanin.

A study of the properties and composition of the pigment is best made in a form from which it may be obtained easily and abundantly. Octopus furnishes such a source. The arterial color of the Octopus is blue, the venous blood colorless. Observation of the living animal shows that the blood in the vessels leading into the gills is colorless, and that the blood in the vessels coming from the gills is blue. According to Fredericq, the change can be easily seen taking place in the blood vessels of the living animal if the large artery going to the head is laid bare. So long as the animal is breathing normally this artery carries blue blood. As soon as the breathing is disturbed, either by taking the animal out of the water or by putting the finger into the mantle opening, the blood immediately begins to lose color and becomes pale. Also blue arterial blood taken

outside the body will lose its oxygen and its color if placed in a vacuum, if it is placed in contact with a live tissue, or if kept for some time in a closed vessel.

Properties of Hæmocyanin.—Hæmocyanin is a respiratory pigment. Harless in 1847 referred to the color of the blood of some of the Molluscs. In Helix he had noticed that the blue color of the blood appeared more clearly if the blood was exposed to atmosphere air, and that the color disappeared if a stream of carbon dioxide was passed through the blood. The blue color would reappear if the blood was again exposed to the air. This was apparently the first recognition that the substance in question might exist in two readily reversible forms, the oxidized and the reduced. The oxyhæmocyanin in freshly drawn blood of *Octopus macropus*, freed from leucocytes by coagulation and filtration, is reduced to hæmocyanin when the blood is spread in a thin layer in a vacuum. In bulk, neither the bubbling through of nitrogen gas nor the presence of a vacuum causes any appreciable discoloration. When the leucocytes are present there is a rapid reduction of the oxyhæmocyanin, the rate and degree depending upon the temperature, number of leucocytes, and the manner of their agglutination. This reduction is due to the respiratory activity of the leucocytes.

Harless and Bibra noted the fact that the blood of the Cephalopods, as well as of the snails, contains copper, and demonstrated the fact that the copper is combined with the coloring matter of the blood. The copper content of the pigment of the animals under discussion has been amply confirmed by later investigators, as will appear presently.

The blue color of the bloods of many of the Crustacea and of Limulus has been noted for a long time. Genth mentioned the blue color of the Limulus and stated that the color was due to the presence of an albuminous substance containing copper. Haeckel in 1857 noticed the change which takes place in the color of the blood of Homola, especially after being exposed to the air, turning from light blue to a very dark purple or almost black.

Jolyet and Regnard noticed that there is among the Crustacea, in addition to the blue pigment, a red one which is sometimes so abundant as entirely to mask the blue pigment. If the blood of one of the crabs is shaken in the air it appears a beautiful blue when viewed by reflected light and brown by transmitted light. If the blood is treated in a vacuum with sodium bisulphite, there is a resulting loss of color, and then the blood turns to a bright rose red. Krukenberg has given special attention to this matter of the appearance of the two pigments and is authority for the statement that red and blue pigments appear in the bloods of most of the Crustacea, but that they appear in very different proportions and relations. In the bloods of Homarus, Maja, and Portunus the blue pigment is in excess, and these bloods color blue or blue green upon

shaking with air, while the bloods of Astacus and some others appear almost colorless or are weakly reddish.

Crystallization of Hæmocyanin.—Hæmocyanin in crystalline form has been obtained from the bloods of various animals by dialysis, electrolysis, supersaturation, and salting out. Although the crystals obtained from the bloods of different forms are dissimilar, it seems too early to conclude that they represent radically different kinds of hæmocyanin in the several bloods. It must, of course, be assumed that the protein molecules of the different forms studied are somewhat different in their architecture and composition. It seems reasonable to expect that among these lower animals there should be hæmocyanins of somewhat different composition and physical properties, just as among higher animals there are to be found hæmoglobins which differ in certain respects from each other. Hæmocyanin from two species of Octopus and of Eledone crystallizes in the form of prisms—often in groups of needles. These crystals are soluble in water, though boiling or the addition of alcohol converts them into an insoluble mass. Recrystallization of the hæmocyanin of Octopus vulgaris produces characteristic, greenish-blue, hemihedral crystals—*projectiles,* which resemble a section of a sugar loaf. A salt-free hæmocyanin solution coagulates at from 68 to 72°C.

Spectral Properties of Hæmocyanin.—Spectrographic studies show that the oxyhæmocyanins of the Gastropod Molluscs, Cephalopods, marine and freshwater Crustaceans, and of solutions of oxyhæmoglobin crystals in 0.01*N* sodium carbonate all give a clear and intense absorption band in the yellow when examined in suitable concentration and solvents (see Fig. 11). This band disappears when the oxyhæmocyanin is reduced. Slight variations in the axes of absorption are considered due to differences in the amount of electrolytes present.

Analyses of Hæmocyanin.—Griffeths, Henze, and Alsberg and Clark have made analyses of the hæmocyanins of Sepia, Octopus, Limulus, and Crustacean blood. Table 13 indicates the percentage composition of these respiratory proteins in the different forms studied.

TABLE 13

	Griffeths, average of Sepia and Crustacea	Henze, hæmocyanin crystals, Octopus	Alsberg and Clark, Limulus
Carbon	54.155	53.66	48.94
Hydrogen	7.095	7.33	7.10
Nitrogen	16.268	16.09	16.18
Sulphur	0.647	0.86	1.56
Copper	0.328	0.38	0.28
Oxygen	21.507	21.68	25.94

It is to be noted that the hæmocyanin of Limulus differs from that of Octopus and of Sepia in its percentage composition. It also differs from them in its precipitability by dialysis, by full saturation with magnesium sulphate and half saturation with ammonium sulphate in not having been crystallized and perhaps in being more sensitive to acid. Alsberg and Clark are decidedly of the opinion that the hæmocyanin of Limulus is somewhat different from that of the other forms mentioned. In general, it may be stated that saturation with ammonium sulphate gives, after a long time, a complete precipitation of hæmocyanin, but saturation with magnesium sulphate gives only an incomplete precipitation. The hæmocyanin solution is also precipitated by dilute acetic acid, the precipitate being redissolved by the addition of an excess of the acid. Running carbon dioxide through the solution also gives a precipitation, though it is incomplete. Since the pigment contains copper, it gives the biuret reaction simply upon the addition of alkali. It shows a typical protein reaction.

The Rôle of Hæmocyanin as an Oxygen Carrier.—The chief interest, of course, in hæmocyanin is its character as an oxygen carrier. It has already been shown in another place that the oxygen content of water taken from the open sea is from 4.8 to 6.3 cubic centimeters per liter at normal atmospheric pressure, and that the water of a freshwater river varies from 6 to 8 cubic centimeters per liter. It is now of interest to find whether the blood which contains hæmocyanin in solution has the power to combine with a larger amount of oxygen than has the water. Griffeths found in the blood of one of the Cephalopods from 12.0 to 14.6 cubic centimeters of oxygen, 29.1 to 32.1 cubic centimeters of carbon dioxide, and 1.2 to 2.0 cubic centimeters of nitrogen per hundred cubic centimeters. This shows that the content of the hæmocyanin-containing blood is greatly in excess of that of sea water or fresh water as far as these important gases are concerned. Cuenot found in the blood of the snail, examined by Schutzenberger's method, 11.5 to 12.8 cubic centimeters of oxygen per liter, and in the Moselle River from 4.2 to 4.5 cubic centimeters of oxygen. These figures, if correct, do not show the same contrast found in the figures given by Griffeths, but do show that the blood even of a form like a snail is capable of taking up more oxygen than the running water in which the animal lives. It need hardly be pointed out that the blood of the snail contains only a small amount of respiratory protein as compared with the bloods of some of the higher molluscs. As a matter of comparison let it be remembered that the blood of the dog contains about 21 volumes per cent of oxygen.

Importance of Copper in Hæmocyanin.—The respiratory capacity of the blood of an Octopus and probably of the Cephalopoda, in general, is relatively very high, three or four times that of the blood of the Helix. Among the Crustaceans none has been found having as high a respiratory

value as that found in Octopus; and among the Brachyura the respiratory capacities are particularly feeble, corresponding approximately to those of Helix. There is a general correspondence between the copper content of the blood and the oxygen-absorbing capacity. The following table indicates the copper content of a number of different bloods:

Table 14

	Milligrams of Copper per 100 Cubic Centimeters of Blood
Helix pomatia	6.5–12.5
Octopus vulgaris	18.0–23.5
Sepia	23.7
Cancer pagurus	3.5–13.5
Maja squinado	3.5
Squilla mantis	6.1
Palinurus vulgaris	7.5–11.0
Carcinus mœnas	9
Homarus vulgaris	9.5–10.5
Astacus fluviatilis	4.0– 8.0

The change in the copper content appears to parallel the variation in the hæmocyanin content of the blood, as determined roughly by the intensity of the blue color. A parallel determination of the copper content and the oxygen absorption by the blood gave the following result:

Table 15

100 cc. snail blood containing	6.5 mg Cu per 100 cc. absorbed	1.45 cc. oxygen
100 cc. snail blood containing	11.5 mg Cu per 100 cc. absorbed	2.2 cc. oxygen
100 cc. lobster blood containing	9.5 mg Cu per 100 cc. absorbed	3.0 cc. oxygen
100 cc. lobster blood containing	10.5 mg Cu per 100 cc. absorbed	3.1 cc. oxygen
100 cc. crab blood containing	8.0 mg Cu per 100 cc. absorbed	2.5 cc. oxygen

Henze found by means of gas analysis of the blood of a Cephalopod a content per 100 cubic centimeters of 3.09 to 3.70 per cent oxygen, 8.11 to 8.74 per cent carbon dioxide, and 1.92 to 3.58 per cent nitrogen. Assuming a hæmocyanin content of 9 per cent, he calculated that 1 gram of hæmocyanin can take up 0.4 cubic centimeter of oxygen. The hæmoglobin of ox blood holds 1.34 cubic centimeters of oxygen per gram. It may therefore be roughly said that hæmocyanin has from one-fourth to one-third the oxygen-carrying power of the hæmoglobin.

Are There Many Hæmocyanins?—Various authors have made studies of the physical properties and chemical composition of hæmocyanins coming from various animal forms. It appears true that the powers of dialysis, forms of crystallization, amount of copper in the molecule, color reactions, and even spectroscopic absorption bands vary in different samples. For example, the hæmocyanin of Limulus differs from that of Octopus in percentage composition, in precipitation by saturated mag-

nesium sulphate, by half-saturated ammonium sulphate, and in not having been crystallized.

The bloods of the invertebrates vary greatly in their capacity as carriers of oxygen and of carbon dioxide. This variation is, of course, correlated with the presence or absence of one or another of blood pigments and the relative amounts of blood pigments found in the blood of different forms. Table 16 shows the amount of oxygen, carbon dioxide, and nitrogen dissolved in the blood of the forms mentioned.

TABLE 16.—TABLE SHOWING THE AMOUNT OF OXYGEN, CARBON DIOXIDE, AND NITROGEN DISSOLVED IN 100 CUBIC CENTIMETERS OF BLOOD IN THE FORMS MENTIONED

Animal	Oxygen	Carbon dioxide	Nitrogen	Amount
Sea urchin	0.8	18.0	15.2	Per liter
Worms	11.0	28.04	1.76	Per 100 cc.
Worms	13.02	30.15	1.96	Per 100 cc.
Sepia	12.9	29.1	1.2	Per 100 cc.
Sepia	14.6	32.1	2.0	Per 100 cc.
Octopus	13.28	30.23	1.29	Per 100 cc.
Octopus	13.65	31.29	1.45	Per 100 cc.
Palinurus	14.29	28.92	1.20	Per 100 cc.
Palinurus	14.76	30.00	1.82	Per 100 cc.
Homarus	14.81	28.84	1.76	Per 100 cc.
Homarus	14.99	31.11	1.85	Per 100 cc.
Coleoptera (average)	16.21	32.92	1.09	Per 100 cc.
Lepidoptera (average)	17.24	34.97	2.35	Per 100 cc.
Cancer pagurus	14.79	27.14	1.01	Per 100 cc.
Cancer pagurus	14.96	28.62	1.30	Per 100 cc.

CHAPTER XI

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE FLUID MEDIUM

It has already been pointed out that a mutual relationship exists between the organism as a whole and the environment in which it carries on its life's processes. It has been shown that in marine organisms, in general, the ocean forms an environment whose qualities are such as to render it, par excellence, the medium for many organisms.

There is sufficient ground for believing that the present land and aerial forms of animals are descendants of forms once living in the sea, and, further, as far as this earth is concerned, living matter had its first abode in what was then the ocean.

Ancestry of the Fluid Medium.—The first living forms had the sea for their environment. Every cell, doubtless, came into contact with this fluid which was at the same time the source of its food and oxygen supply. As cells began to be associated in smaller or larger masses, channels were left between them through which the water of the sea might find passage. Animals a little farther along the scale of development shut off their body cavities, vascular and otherwise, from direct communication with the sea, but did not succeed in freeing the cells from the necessity of getting their food and oxygen supplies by diffusion from a solution. The fluid shut within the animal body furnished the immediate environment of the cells; it took the place of the sea in the economy of the organism. It has been seen that the fluid becomes more and more complex in its structure as one passes in review from the lower to the higher forms. And while the internal fluid sea, bathing the individual cells, has become in some respects more complex and able to play a greater variety of functions in the life of the organism, there are certain features in which it harks back to the primitive conditions which must have existed millions of years ago.

Freezing-point Determinations of Body Fluids.—The osmotic pressure of the body fluids of various animals may be determined through obtaining the depression of the freezing point of the liquid as compared with that of distilled water. Table 17, compiled from data given by G. G. Scott in a paper in the *American Naturalist*, and also in New York Academy of Science, and from other data indicates the facts as they are at present known. The Greek letter Δ is used to indicate *depression of freezing point.*

TABLE 17

Species	Blood Δ	Osmotic pressure, atmospheres	Water Δ	Locality
Cœlenterata:				
Alcyonium	2.195	26.31	2.29	Naples
Echinodermata:				
Astropecten	2.312	27.75	2.29	Naples
Asterias	2.295	27.57	2.29	Naples
Holothuria	2.315	27.81	2.29	Naples
Annelida:				
Hirudo	0.43	5.182	0.03	Fresh water
Arthropoda:				
Homarus vulgaris	2.292	27.53	2.29	Naples
Maja squinado	2.36	28.34	2.29	Naples
Homarus americanus	1.82	21.88	1.80	Woods Hole
Limulus polyphemus	1.90	22.84	1.82	Woods Hole
Astacopsis	0.615 (av.)	7.41	....	Fresh water, Australia
Astacus	0.80	9.635	0.03	Fresh water
Dytiscus marginatus	0.95	11.44	....	Fresh water
Acilius sulcatus	0.49	4.70	....	Fresh water
Mollusca:				
Aplysia	2.31	27.75	2.29	Naples
Octopus	2.24	26.91	2.29	Naples
Anodonta	0.15	1.81	0.03	Fresh water
Unio	0.13	1.568	0.03	Fresh water
Cyclostomata:				
Polistrotema	1.966	23.62	1.924	Monterey
Elasmobranchiata:				
Mustellus vulgaris	2.36	28.34	2.29	Naples
Trigon violacea	2.44	29.30	2.29	Naples
Scyllium	2.31	27.75	2.29	Naples
Torpedo	2.292	27.53	2.29	Naples
Acanthius vulgaris	1.90	22.84	1.91	North Sea
Carcharias littorina	2.03	24.40	1.82	Woods Hole
Mustelus (average 80 specimens)	1.869	22.47	1.81	Woods Hole
Squalus acanthias	1.70	22.40	1.42	New York
Pisces:				
Charax	1.04	12.52	2.29	Naples
Serranus	1.035	12.46	2.29	Naples
Oncorhyncus	0.762	9.174	1.924	Monterey
Pleuronectes	0.71	8.552	1.91	North Sea
Lophius	0.80	9.635	2.00	Arachon
Tautogaonitis	0.86	10.36	1.82	Woods Hole
Conger eel	0.82	9.875	1.82	Woods Hole
Scup	0.75	9.033	1.82	Woods Hole
Perca	0.507	6.109	0.03	Fresh water
Pleuronectes	0.68	8.191	0.03	Fresh water
Salmo	0.567	6.838	0.03	Fresh water
Barracouta	0.979	11.79		
Thyrsites	0.978	11.78		
Amphibia:				
Rana esculenta	0.563	6.787		
Rana ? (spring frog)	0.38–0.40	4.580–4.821		
Bufo	0.761	9.154		
Reptilia:				
Emus europa	0.463	5.583		
Emydura (av. 3 sp.)	0.560	6.747		
Emydura (av. 3 sp.)	0.548	6.379		
Aves:				
Capon	0.66	7.951		
Turkey	0.75	9.033		
Larus	0.69	8.312		
Uriatroile	0.66	7.951		
Eider	0.65	7.830		
Fulmarus glacialis	0.65	7.830		
Mammalia:				
Porpoise	0.74 (?)	8.913		
Dolphin	0.83 (serum)	9.995		
Baleen whale	0.70 (?)	8.432		
Phoca barbata (adult)	0.64	7.710		
Phoca barbata (young)	0.66	7.951		
Phoca fœtida	0.71	8.552		
Horse	0.58	6.988		
Ox	0.601	7.304		
Rabbit	0 57	6.807		
Rabbit, Australia	0.59	7.108		
Sheep, Australia	0.59	7.108		
Echidna, hibernating	0.624	7.493		
Echidna awake	0.600	7.299		
Man	0.560	5.747		

The figures indicating the osmotic pressures are for 0°C. The osmotic pressures increase by $\frac{1}{273}$ for each degree rise in temperature, or 0.00367 of pressure at 0°. For example, the osmotic pressure of human blood at 37° is $\frac{0.56}{1.87} \times 22.40 \times 0.00367 \times 37$, which is 0.9109; 6.747 plus 0.9109 gives the osmotic pressure of human blood at 37°.

Chemical Composition of the Bloods of Invertebrates.—For some reason comparatively little work has been done to make clear the chemical composition of the body fluids of invertebrates. Several analyses giving the ash composition of certain bloods have been published, but there has been little evidence as to the form in which the various elements may have been combined in the blood. R. G. Myers has given results of analyses giving total nitrogen, non-protein nitrogen, urea and ammonia nitrogen, preformed and total creatinine, uric acid, creatin, sugar, cholesterol, chlorides—as sodium chloride—calcium—as calcium oxide—total solids, and ash. Qualitative tests included the reactions to litmus, the biuret, Millions, Hopkins-Cole, the xanthoproteic tests, and that for loosely combined sulphur.

The values given in Table 18 exhibit several differences when compared with similar determinations made upon the bloods of higher forms. The exceptionally high content of inorganic salts, 2900 to 3200 milligrams, and the low protein nitrogen content, 0.7 to 340 milligrams per 100 cubic centimeters of the blood, may be taken in illustration.

The low protein content of the invertebrate blood undoubtedly bears a causal relation to its low viscosity. Compared with the viscosity of the bloods of more highly developed marine forms and mammals, it should be lower. In the mollusc and crustacean blood the increased protein content would be paralleled with an increased viscosity, and this, in turn, is associated with an improved cardiac and circulatory mechanism.

Myers contends for the buffer effects of the protein content of the invertebrate blood in maintaining the required hydrogen-ion concentration. The values stated by Myers (pH 6.4 to 6.7) are certainly unreliable in view of the fact that they were determined by litmus. It would also seem that the very small amount of the proteins present would hardly be sufficient to play the rôle suggested. Some other way of maintaining the proper concentration must be present.

Reference to the table will show that the urea content is relatively low, though the mean value is higher than that observed in some of the marine and freshwater fishes. The ammonia, preformed creatinine, and creatin are generally lower in the bloods of invertebrates than in the bloods of fishes and mammals. A large part of the non-protein nitrogen is undoubtedly that contained in the amino acid. Sugar is found in relatively large quantities in the blood of every invertebrate studied. In comparison with the sugar in the bloods of other forms the differences are

TABLE 18.—TABLE SHOWING RESULTS OF ANALYSES OF BODY FLUIDS OF CERTAIN INVERTEBRATES. (*R. G. Myers*)
All weights in milligrams

	Specific gravity	Solids	Ash	Ca as CaO	Cl as NaCl	Total N	Non-protein nitrogen	Urea + NH_3N	Urea	NH_3N	NH_3 Amino N	Creatinine				Sugar	Cholesterol
												Preformed	Total	Creatin	Uric acid		
Jellyfish (*Phacullophora*)	.....	....	....	...	.....	44	12	5	.....	.....	...	0.01	0.30	0.29	.....	109	
Starfish (*Pisaster ochracea*—5-rayed)	.....	....	....	53	3225	5	4.4	1.0	.....	0	...	0.01	0.13	0.12	.....	...	0.9
Starfish (Picnopodia h.—20-rayed)	.....	....	....	56	3225	6	4.0	2.0	.....	.....	...	....	....	....	.....	29	
Sea urchin (Strongylocentrotus)	1.024	3485	2992	63	3140	12	8.6	1.0	0.92	0.08	...	0.11	0.27	0.16	Trace	61	4.0
Clams (*Saxidomus nuttali*)	.....	4330	2800	307	.....	44	11.0	10.0	9.05	0.95	...	0.28	0.74	0.43	.....	77	
						59	13.0	....	....	....	...	0.31					
Schizotherus nuttali	1.029	4208	3290	193	3190	40	10.0	3.3	2.94	0.66	8.0	0.38	0.80	0.42	Trace	74	2
							14.0	3.6									
Cryptochiton stelleri	1.030	.....	....	...	.....	197	....	3.0	2.00	0.40	...	....	....	....	.....	41	
Cryptochiton stelleri composite	.....	....	....	.....	.....	231	25.0	4.0									
Abalone (*Haliotis rufescens*—black)	1.029	5154	2376	74	3130	231	23.3	2.4	2.27	0.13	...	1.25	2.08	0.83	.....	91	3.6
		4730	....	87	3160	168	15.0	1.4	1.11	0.29	...	0.31	0.78	0.47	Trace	61	3.0
Crab (two species—*Cancer productus; Cancer antennarius*)	.....	4685	....	73	2895	358	18.0	6.2	5.15	1.05	...	0.20	0.78	0.58	47	90	5.7

not great. Cholesterol appears in very small quantities, the amount being much less than in the blood of man. The combined amino acids, tyrosine, cystine, and tryptophane—the two latter in considerable quantities—were shown to be present in most of the bloods, and in one case in the tissues, of the invertebrate animals studied. In view of the wide range of animal forms over which these observations were made, it would seem very likely that similar results might be expected if other forms should be examined.

TABLE 18*a*.—QUALITATIVE DATA ON SAMPLES OF WHOLE BLOOD

Animal	Color of blood	Reaction					
		Litmus	Xantho-proteic	Millions	Hop-kins-Cole	Lead	Biuret
Jellyfish (*Phacullophora camtschatica*)	Colorless macerate	Slightly alkaline	Medium	.....	Quite strong	Weak	Medium
Cryptochiton.....	Dark amber	Slightly alkaline					
Strongylocentrotus.....	Orange	Slightly alkaline	Medium	Medium	Weak	Weak	Medium
Haliotis rufescens.....	Light indigo blue	Slightly alkaline	Quite strong	Quite strong	Strong	Very strong	Pinkish
Schizotherus.....	Bluish tint	Slightly alkaline	Medium	Medium	Weak	Quite strong	Medium
Cancer productus and antennarius	Light greenish blue	Slightly alkaline	Strong	Quite strong	Very strong	Strong	Pinkish

Correspondences between Body Fluids and Sea Water.—The characteristics of the internal fluids of animal forms and the sea correspond along four lines which should receive comment:

1. The correspondence as to molecular concentration resulting in osmotic pressure.

2. The correspondence as to the relative concentration of the various inorganic salts and ions present in both sea water and body fluids.

3. The correspondence in temperature.

4. The correspondence in hydrogen-ion concentration.

1. Molecular Concentration and Osmotic Pressure.—From an examination of the facts presented in Tables 17 and 19 the following conclusions may be drawn.

a. The body fluids of the marine invertebrates, Cyclostomes, and Elasmobranchs have body fluids of slightly greater osmotic pressure than the sea water in which they live. This slight difference is so small as, apparently, to have been overlooked by various authors dealing with the subject.

b. The body fluids of freshwater invertebrates have freezing points considerably less depressed, their bloods varying a great deal from phylum

to phylum but in no case being more concentrated in salts than that of the freshwater fishes.

c. The bloods of marine fishes have osmotic pressure somewhat less than one-half that of the sea water in which they live.

d. The bloods of freshwater fishes, amphibia, and reptiles have salt concentrations of about the same values or slightly less than those of the land mammals.

e. The bloods of all the land mammals, as far as examined, have osmotic pressures of nearly the same value. The marine mammals show higher values, corresponding more nearly to the osmotic pressures of the marine fishes living under similar conditions. The osmotic value of the blood of the seals takes a position somewhat between those of the freshwater and marine fishes.

An analysis of all the facts seems to indicate that there is some causal relation existing between the osmotic pressure of the sea water and the osmotic pressures of the bloods of various animal groups. Undoubtedly, life originated in the sea in water which was relatively dilute as compared with the sea water of the present time. This sea water became more and more concentrated as various salts were washed down from the land into the sea. At various periods some of the animals living in the sea migrated up the streams and came to live in water which was less concentrated in salts, others emerged from the sea and began to live upon the land. Those which remained in the ocean have been for generations continuously subject to the gradually increasing concentration of salts in the sea water. Figure 16 presents in graphic form a report of some of the facts concerning the osmotic pressures of the body fluids of various animal groups, and a suggestion is offered as to a possible explanation of some of these facts. It will be noted that in this figure depressions of the freezing points of bloods and of the sea water are indicated as abscissæ and that duration of time is indicated as ordinates. The freshwater molluscs show a depression of the freezing point, which indicates either that they migrated into fresh water in very early times or that they have developed little power to maintain a salt concentration in the body fluid different from that of their environment. The freshwater worms show a decided increase in the salt content of the blood. The reptiles show a salt content slightly higher than that of the freshwater worms. The freshwater fishes, the mammals, the Amphibia, the birds, all hold salt concentrations of very closely the same value, which slightly overlap each other and which are only slightly below the salt concentration of the bloods of the marine bony fishes taken from the north Atlantic. It seems entirely possible that the ancestors of these forms so closely grouped must have emerged from the waters of the sea at approximately the same period as far as salt concentration of the sea was concerned. The mammals and birds have apparently attempted to maintain in their body

fluids through all the succeeding generations approximately the same concentration as those to which the ancestors were accustomed in their

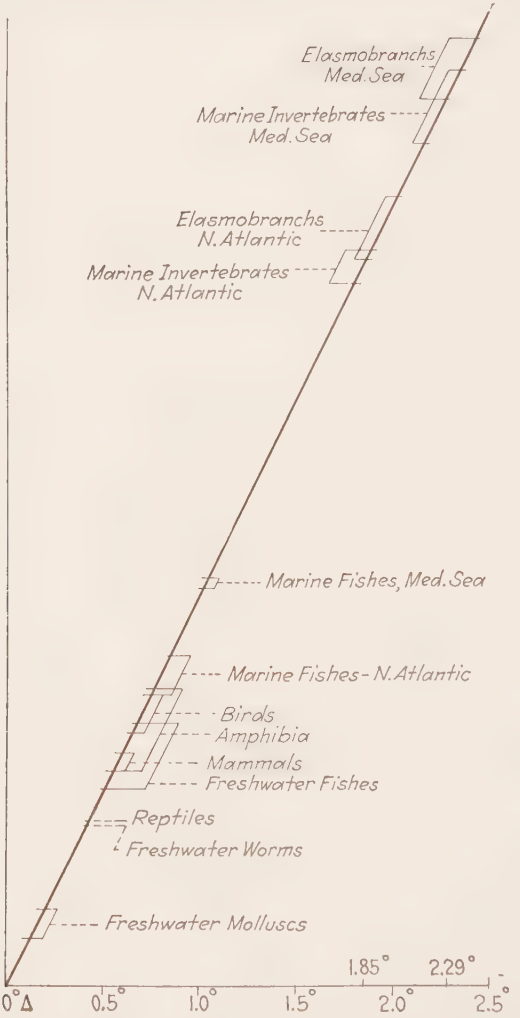


FIG. 16.—A chart indicating the salt content of the bloods of large groups of animals as determined by the freezing-point method. Abscissæ indicate freezing points; ordinates indicate durations of time during which the salt content of the ocean has been increasing from a minimum to the values now found in the Atlantic and in the Mediterranean Sea. The suggestion is made that the grouping of freshwater fishes, mammals, amphibia, and birds may have some reference to the concentration in salts of the sea when the ancestors of these animal groups left their marine habitat. The diagonal may represent the rather steady and continuous increase in salt concentration of the sea water with the passage of time.

original marine habitat. The freshwater fishes and the Amphibia show a somewhat wider range of salt concentrations than do the birds or mammals. This condition can perhaps be accounted for by the fact that

these animals have for the most part lived continuously in an aquatic environment. They have maintained with more or less exactitude the salt concentration in the bloods of their ancestral forms, concentrations which were presumably very similar to the concentration of the sea water in which they lived. The marine fishes both of the open ocean and of the Mediterranean show osmotic pressures a little less than one-half of the concentration of the water in which they lived. This value is subject to considerable modification, as will be shown in later paragraphs. The body fluids of the Elasmobranch fishes, on the other hand, have salt concentrations which are on the average slightly in excess of the salt concentrations of the water in which they live. The bloods and body fluids of the marine invertebrates, however, have salt-concentration values which equal or are very slightly in excess of the concentration of the sea water. Just how far it is justifiable to seek to explain the salt concentration of the bloods of all land forms upon this basis is, of course, somewhat uncertain. It appears in each case that the blood of the animal is in a condition of equilibrium with the external medium, even though it may differ very much from it. It will now be shown that any change in the osmotic condition of the external medium brings about a corresponding and compensating change in the osmotic value of the blood, to a greater or less degree in the different groups, until a new equilibrium is established.

Experiments upon some marine invertebrates (crabs and the worm Arenicola) indicate that in these forms no permanently harmful results follow when they are transferred for a short time to fresh water from the salt water in which they have been living. On being transferred back to the sea water, they will lose the excess water which has been absorbed during their stay in the fresh water. When the normal equilibrium existing between the blood and the sea water is disturbed by placing an animal in either a hypotonic or a hypertonic solution, there is an attempt on the part of the animal to establish a new equilibrium level. It appears likely that the close approximation of the osmotic pressure of the sea water and of the body fluid of the marine invertebrates is not simply a matter of osmosis or diffusion through the animal membranes, but is due to a definite equilibrium established by the boundary cells. Such a point of view certainly has some force when it is realized that the concentrations of the different salts on the two sides of these membranes are usually slightly different.

As an example of the change resulting from a transfer from sea water to fresh water, observations made upon the eel may be mentioned. Specimens taken from the sea off Helgoland contained blood which depressed the freezing point 0.649°C. Several specimens were transferred to slowly freshened water and the blood tested after 3 days. The depression of the freezing point in these specimens was 0.582°C. Speci-

mens which had been for a long time in fresh water showed a depression of 0.57°C.

When living matter first appeared in the water of the sea it was undoubtedly adjusted to the salt content of the water as it existed at that time. The salt content was being continually increased through the addition of salts which were washed down from the land into the ocean. These additions must have accrued very slowly indeed. The fact that the living organisms continued to exist is itself an indication of the fact that there was a continual adjustment of the organisms to the increased salt concentration. This adjustment has gone on until it is now discovered that organisms have become adapted to living in a salt solution the concentration of which may be equal to that of a $\frac{5}{8}$ *M* NaCl solution, or even more highly concentrated.

But it will be noted that not all animals lived in the sea. At different periods in the history of animal forms there have been migrations from the sea and the taking on of new environmental relations. Animals which had been living in the sea moved up into the fresh waters, or at least into waters less salt than those of the ocean, or they moved out upon the land and took up a new habit of life. In either case there were significant physiological adaptations to the new habit of life. In certain cases the respiratory system was fundamentally altered, and undoubtedly other systems of organs met with conditions calling for some radical change in the method of operation. The cells of the bodies of these forms had become accustomed to association with an environmental liquid having a certain total concentration and possessing certain definite chemical characteristics. If life in the cells of these migrating organisms was to be preserved, it was necessary that this relationship between the cells and their immediate environment be continued. It is thus found that all of the land forms possess body fluids having not only a definite concentration, but a concentration which seems likely to be about that of the sea at the time of the migration from the sea to the fresher water, or to the land.

The mammals, then, possess body fluids having about the concentration of the sea at the time when the ancestors of the present land forms left the sea. Dr. Macallum has estimated the salt content of the sea during the Cambrian period and finds a figure which corresponds very closely with that of the blood of the present land forms.

It has been customary to think and to speak of living organisms as exceedingly plastic structures. In a sense this is true, but in another sense it is not. There can hardly be imagined a condition more definitely determined and fixed than the relation which exists between the concentration of body fluid and that of the medium in which the animal lives. The constancy with which the saline concentration of the

cellular environment has been maintained through millions of years is certainly a fact worthy of remark.

2. The Correspondence between the Relative Concentration of Salts in Body Fluids.—In considering the total effects of the salts of the blood, the proportions in which the different salts appear in the blood have been left out of consideration. It has been found that the total salt content of the blood of animals living in the sea is in many cases almost the same as that of the sea water. If, now, an analysis is made of the salts found in the blood, it will be seen that there is a very striking similarity between the salts of the blood and the salts of the sea water. The conclusion cannot be escaped that there is a causal relation between the two sets of facts. Indeed, Macallum makes the claim that there is a very strong similarity between the salt content of the blood of mammals and that of the sea during the pre-Cambrian period. In passing from the marine invertebrates up the branches of the phylogenetic tree, there is found step by step a lowering of the osmotic pressure of the blood as a whole and a marked diminution of the magnesium content of the blood. At the same time there is a marked increase in the concentration of the potassium. This would indicate that the membranes of the cells lying between the body fluid and the sea are not readily permeable to magnesium and potassium ions, or, if permeable, more permeable in one direction. It would also point to the conclusion that the concentration of these substances as now found in the sea is different from that to which the protoplasm of the cells was originally adapted, and also that the present concentrations of these substances in the sea as compared with the concentration of sodium salts are not the optima concentrations for a very large number of living forms.

A glance at the figures shown in Table 19 will give an idea of the ratios of the concentrations of the principal substances found in the sea water and in the body fluids of several widely separated forms.

Church has called attention in a very interesting manner to some facts in regard to the water of the sea. There is still water enough upon the surface of the earth to form a layer about 2 miles in thickness over the whole surface of the globe. The mass of liquid is about 1.3×10^{18} tons. Of this, about $3\frac{1}{2}$ per cent consists of salts in solution. It has already been pointed out that electrolytes in solution tend to dissociate into ions. This is true of the salts of the sea water. The water of the sea may be considered a solution containing eight elementary ions, four electropositive (cations)—Na', K', Mg'', Ca''—and four electronegative—(anions) Br^-, Cl^-, SO_4^{--}, and CO_3^{--}—also traces of N, Ph, I, Fe, Mn, Ag, etc. The general run of the elements follows the sequence of the periodic law from twelve to forty, in which aluminum is deficient.

The solutes of sea water are ionized nearly to the extent of 90 per cent. The water itself is ionized to the extent of 10^{-14} normal. The water of the

sea is approximately neutral, ranging in pH from 7.9 to 8.2. It may be said that the sea water is by virtue of its ionization non-molecular to the extent of about 3 per cent by weight. This commonplace medium hitherto regarded as a molecular fluid must be considered as an ionic organization of inconceivable complexity. There is not, at the present time, any means of knowing the various ways in which the various ions present may relate themselves to each other in the sea water. Figure 17 is an attempt to show in a graphic way how some of the principal ions of the sea water may become related to each other.

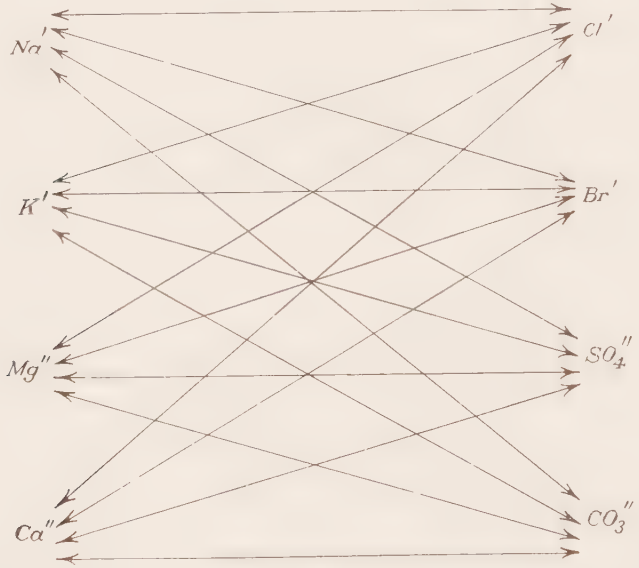


FIG. 17.—Diagram to indicate possible ionic combinations in sea water.

TABLE 19.—TABLE SHOWING RELATIVE CONCENTRATION OF PRINCIPAL IONS IN THE BLOODS OF THE ANIMALS MENTIONED

	Na	K	Ca	Mg	SO_3	Cl
Sea water	100	3.613	3.911	12.106	20.9	180.9
Aurelia flavidula	100	5.18	4.13	11.43	13.18	185.5
Limulus polyphemus	100	5.62	4.06	11.20	13.33	186.9
Homarus americanus	100	3.73	4.85	1.72	6.67	171.2
Acanthias (dogfish)	100	0.461	2.71	2.46	.....	165.7
Gadus (cod)	100	9.506	3.93	1.41	.....	149.7
Pollock	100	4.33	3.10	1.46	.....	137.8
Dog	100	6.86	2.52	0.81	.....	128.5
Mammal, average	100	6.69	2.58	0.80	.....	118.3
Man	100	9.22	3.37	1.76	.....	101.4

The figures presented in the second part of the table would seem to indicate that the present ionic ratios are only slight modifications of the ratios which prevailed in the blood sera of the ancestors of the vertebrates. The high proportion of magnesium in the dogfish, cod, and pollock may be explained as due to the influence of the sea water, which is especially rich in this element, for the Elasmobranchs have been oceanic since their origin in the Silurian at the latest, and the Gadidæ (cod and pollock) have been resident in the ocean since the origin of Teleosts from a ganoid form in the Cretaceous. This long association with the sea has given 1.77 per cent of total salts in the dogfish, 1.25 in cod and pollock, while in mammals the total salts comprise about 0.9 per cent.

Another set of figures taken from another source indicates something of the same character. These figures give the percentage composition of the ash of the body fluids of the forms mentioned as compared with sea water.

TABLE 20

	Echinoderms	Sabella	Mollusca	Crustacea	Insects	Sea water
MgO	1.05– 1.38	1.22	1.51– 1.86	1.88– 1.91	1.00– 1.16	6.2
K_2O	4.52– 4.81	4.03	4.80– 4.92	4.77– 4.97	4.06– 4.20	1.33
Na_2O	43.78–44.22	45.23	43.9 –45.4	4.39– 4.99	43.29–44.03	41.2
P_2O_5	4.24– 4.62	4.56–	4.53– 4.90	4.81– 4.90	3.21– 3.61	
SO_3	2.22– 2.36	2.10	2.66– 2.83	2.75– 2.90	2.80– 2.93	6.4
Cl	39.63–40.40	39.26	35.55–38.16	36.96–37.65	41.30–43.16	55.3
CaO	.....	3.42	2.31– 3.72	3.54– 3.79	2.06– 2.43	1.67

The figures of this table appear to justify the following conclusions:

1. The same salts are present in the body fluids as are present in the sea water.

2. For some reason there is a tendency on the part of the forms examined to exclude a portion of the magnesium of the sea water.

3. There is a similar tendency on the part of the organisms to store up potassium. This substance may be in combination with some of the colloids of the body fluid and so not be in a condition in the blood to follow the usual laws of osmotic exchange.

4. The membranes of the cells are far from being perfectly permeable to the salts of the sea water.

Physiologically Balanced Saline Solutions.—In laboratory investigations and in hospital practice it has been found desirable to substitute in place of the blood a solution which would permit the tissues under observation to act in a normal way. Such solutions must contain the salts found in the blood in approximately the same molecular concentration and the same relative proportions. As has already been pointed out, the sea water possesses a composition very similar to that of the bloods of

many of the invertebrates as far as the proportions of the most important electrolytes are concerned. This has led to the idea that the sea water may be considered as a physiologically balanced solution, and used in place of a body fluid in experiments upon marine forms. Such a use of the sea water can easily be proved possible if certain slight but important changes are made in its composition, as, for example, the addition of a small amount of a calcium salt. As has been pointed out, sea water is an exceedingly complex solution, and it is only after a great deal of effort that biological chemists have finally succeeded in elaborating a formula for an artificial solution which has the same properties as the water of the open ocean as far as its effects upon living matter are concerned. McClendon has given the following formula as representing the best information obtainable at the present time regarding the composition of this solution:

TABLE 21.—McCLENDON'S SEA WATER
All solutions are of $M/1$ concentration unless otherwise stated

$NaCl$	483.65 cc.	Na_2SiO_3	0.0025 gr.
KCl	10.23 cc.	$Na_2Si_4O_9$	0.005 cc.
$MgSo_4$	28.55 cc.	H_3PO_4	0.002 cc.
$MgCl_2$	25.16 cc.	H_3BO_3	1.00 cc.
$CaCl_2$	11.00 cc.	Al_2Cl_6	0.01 cc.
$NaBr$	0.8 cc.	NH_3	0.001 cc.
$NaHCO_3$	2.5 cc.	$LiNO_3$	0.002 cc.
		H_2O	373.63 cc.

In a solution of the composition just stated, even the most delicate marine algæ will live for long periods in an apparently normal way. Any change in the proportions of the various substances present in the sea water or in the physiological saline will make its impress upon the physiochemical system, resulting in a change of balance. This change of balance will be indicated by a modification in the behavior of the organ or tissue under observation. The change of balance may be physical or chemical or both. If the ratio between the Na, K, Ca elements of the solution be modified, there may be changes in surface tension, as shown by Dr. Clowes, and it is likely that balances existing in the ion protein compounds within the cells may be upset, as contended by Dr. Loeb.

While it is true that the sea water is an exceedingly complex solution, and while it is true that the body fluids of animals are at least as complex as the sea water, it has been found that a much simpler form of solution will serve as a protective medium for use during experimentation if care is taken to provide for the correct proportions of the more important saline constituents of the natural medium. Such a fluid would not serve for the preservation of normal conditions extending over a long period

of time, but will serve for relatively brief studies. Van't Hoff has given us the formula for such a solution.

Van't Hoff's Solution.—Van't Hoff's solution contains the principal salts of the sea water in the molecular proportions in which they are to be found in the sea water. The solution may be made of any desired concentration, for example, $M/1$, and then diluted to the same concentration as that of the sea water in which the animals to be experimented upon normally live. The proportions in which the different molecules occur are as follows: 100 molecules NaCl, 7.8 molecules $MgCl_2$, 3.8 molecules $MgSO_4$, 2.2 molecules KCl, and from 1.5 to 2.5 molecules $CaCl_2$. To this should be added a trace of sodium bicarbonate solution to remove any possible acidity.

It has been found that such a solution will serve as an admirable medium for the study of the action of the heart, and will serve to keep the hearts of certain marine invertebrates beating rhythmically for many hours. This appears not to be true of all of the marine invertebrates, however. As an example of this fact may be cited one of the spider crabs of the Atlantic Coast whose heart will not beat in a solution constructed upon Van't Hoff's formula, but will beat for a long time in a solution containing 100 cubic centimeters M 2 NaCl, 4 cubic centimeters M 2 $MgCl_2$, 8.3 cubic centimeters M 2 KCl, 5 to 6.5 cubic centimeters M 2 $CaCl_2$, and 1.0 cubic centimeter M 10 $NaHCO_3$. The reason for this difference in molecular composition has not been investigated and is not known.

Similar solutions for use upon the tissues of vertebrates have been worked out. They are generally stated in terms of percentages instead of relative molecular values.

TABLE 22

	NaCl	KCl	$CaCl_2$	$MgCl_2$	$NaHCO_3$	NaH_2PO_4	NaHPO	Urea
Ringer's (frog)	0.65	0.014	0.012	....	0.02	0.001		
Gothlin's (frog)	0.65	0.01	0.065	....	0.1	0.008	0.009	
Locke's (mammal)	0.9	0.042	0.024	....	0.01–0.03			
Tyrode's (mammal)	0.8	0.02	0.02	0.01	0.1	0.005		
Fuhner's (shark)	2.0	0.01	0.02	....	0.02	.....	.....	2.5

The Ringer solution given in Table 22 corresponds very closely to a solution built upon the molecular plan as follows:

TABLE 23

100 cubic centimeters $M/9$ NaCl
1.5 cubic centimeters $M/9$ KCl
1.0 cubic centimeter $M/9$ $CaCl_2$
Trace of $NaHCO_3$

There will be found in various textbooks statements that the blood of the frog has about the osmotic pressure of a 0.6 per cent NaCl solution and that human blood has about the osmotic pressure of a 0.9 per cent NaCl solution. As a matter of fact, the freezing-point determinations indicate that the blood of the frog is subject to considerable variation in concentration due to seasonal or other factors. In general, it has been found in the author's laboratory that solutions of *M* 8 concentration furnish an admirable medium for use with the tissues of the frog; of *M* 7 concentration for use with the turtle; and of $^{1}/_{65}$ molecular concentration for use in diluting human blood.

A solution which has proved of considerable value in the study of the heart of the turtle has the composition:

TABLE 24

100 cubic centimeters *M*/7 NaCl
2.2 cubic centimeters *M*/7 KCl
1.7 cubic centimeters *M*/7 $CaCl_2$
Trace of Na HCO_3

Mines' solution for use with frog tissue has the composition:

TABLE 25

100 cubic centimeters *M*/8 NaCl
2.5 cubic centimeters *M*/8 KCl
2.0 cubic centimeters *M*/8 $CaCl_2$
1.5 cubic centimeters *M*/8$NaHCO_3$

It will be noted that the relative proportions of the sodium, potassium, and calcium in this solution are essentially the same as those in which they exist in the sea water.

For the convenience of the student in estimating the strength of salt solutions the following table is included:

TABLE 26

A saline solution containing 0.60 per cent NaCl is 10/97 *M* concentration.
A saline solution containing 0.65 per cent NaCl is 10/90 *M* concentration.
A saline solution containing 0.70 per cent NaCl is 10/84 *M* concentration.
A saline solution containing 0.75 per cent NaCl is 10/78 *M* concentration.
A saline solution containing 0.80 per cent NaCl is 10/73 *M* concentration.
A saline solution containing 0.85 per cent NaCl is 10/69 *M* concentration.
A saline solution containing 0.90 per cent NaCl is 10/65 *M* concentration.
A saline solution containing 0.95 per cent NaCl is 100/615 *M* concentration.

Importance of Metallic Ions in the Blood.—The blood has been shown to be a substitute for sea water, containing the same salts in nearly the same proportions as those in which they are found in the sea. The total

concentration appears to have some relation to the time at which the ancestral form left the sea, in the case of those forms which are not now living in the sea water.

Of the salts which are found in the sea and the blood, by far the most important appear to be the chlorides of sodium, potassium, and calcium.

a. Sodium.—The sodium found in the blood functions chiefly in maintaining the requisite osmotic pressure of the solution. It probably acts also as a cell stimulant, or furnishes a condition necessary for stimulation, especially to be noted in the case of skeletal and cardiac muscle. It exerts in many cases a distinct poisonous effect, acting particularly upon cell membranes.

b. Potassium.—It tends to neutralize a poisonous effect upon protoplasm of calcium ions. It is especially important because of its radioactivity, and may in certain instances at least be replaced by the salts of other radioactive metals, such as caesium and rhubidium, when introduced in equivalent radioactive concentrations. It appears to control quickness of response to stimulation.

c. Calcium.—It is antagonistic to sodium, acts to preserve cell membranes, and regulates the permeability of the membranes.

3. The Correspondence in Temperature between the Sea and Living Animals.—It is probably true that the temperature of the blood of any animal is significant chiefly in the fact that it gives an indication of the temperature of the animal body. The blood undoubtedly receives its heat from the tissues in which heat-liberating, metabolic processes take place. It is shown in another place that the temperatures of the bodies of the so-called cold-blooded animals approximate very closely that of the medium in which they are placed, and that the temperatures of their bodies change with the changes of temperature of the environment.

It has been suggested that certain animals have found it desirable to maintain very constant body temperatures so as to maintain a very constant relation between the various physico-chemical processes which are taking place within the organism, even though the temperature of the environment is subject to modification. Animals making a transfer from one environment to another would appear to be especially likely to profit by such a constant-temperature regulation. At any rate, following the migration from the sea of the ancestors of the present-day warm-blooded animals there were developed mechanisms for the regulation of body temperature. These mechanisms were at the outset very crude and inefficient, and subject to break-down under extreme conditions. As will be found in another section in this work there exist at the present time among some of the less specialized of the warm-blooded animals regulatory systems which are far from perfect. Among the highest forms, however, these regulatory systems have attained a high degree of sensi-

tivity and efficiency. It may be assumed that in the development of these mechanisms an attempt has been made to maintain in the animal bodies about that temperature to which the ancestral forms had been accustomed, or which they had found most advantageous in their original marine environment. It appears possible that there is in the body temperature of the birds and mammals an indication of the temperature of the sea at the time when the ancestral forms left the sea. Another fact of peculiar interest is the diurnal change of temperature in the warm-blooded animals and in the sea. The body of the warm-blooded animal reaches its highest temperature normally between 2 and 6 p.m., and then slowly cools, reaching its minimum between 2 and 6 a.m. The difference is not great, being about 1°C. A similar daily variation in temperature is known to occur in the sea.

4. The Correspondence in Hydrogen-ion Concentration between Body Fluids and Sea Water.—For a long time it has been taught that the blood is a neutral or slightly alkaline fluid. The measurement of the acidity or alkalinity of such fluids has not been until recently an easy matter to accomplish. Recently developed methods, making use of hydrogen electrodes or carefully standardized, very delicate, chemical indicators, have made it possible to determine the hydrogen-ion concentration of liquids to within a comparatively small error. It is well known that distilled water is subject to ionization when it is allowed to stand. In such water an equal number of H ions and OH ions are found. The water is then equally acid and alkaline in character. The number of molecules dissociated in the case of pure water gives a concentration of each ion equal to the 10^{-7} normality (one ten-millionth normal) at a temperature of from 22 to 23°C. If either one of the ions be increased in concentration, a corresponding decrease occurs in the number of the opposing ions. The product of the concentrations is always about 10^{-14} at 22 to 23°C. Instead of writing the concentration in the form just given, it has become customary to use the shorter nomenclature of Sørensen, who has advocated the use of the negative exponent of 10 as a positive whole number, using the symbol pH to indicate that the hydrogen-ion potential exponent is being considered *i.e.*, the exponent of the hydrogen-ion concentration is the common logarithm of the reciprocal value of the normality in hydrogen ions. Thus

$$C_H \text{ (hydrogen-ion concentration) } 0.35 \times 10^{-7} = \text{pH } 7.45$$

Sørensen's Nomenclature.—In order to facilitate the student's use in nomenclature and to make apparent the significance of the terminology, there is here given a brief table of the pH values and the corresponding values of hydrogen-ion normality which are likely to be used most extensively by the physiologist.

TABLE 27.—TABLE SHOWING CONVERSION OF pH VALUES INTO HYDROGEN-ION CONCENTRATION VALUES

pH 6.0 = 1 $\times 10^{-6}$ nH	pH 7.0 = 1 $\times 10^{-7}$ nH	pH 8.0 = 1 $\times 10^{-8}$ nH
pH 6.1 = 0.79 $\times 10^{-6}$ nH	pH 7.1 = 0.79 $\times 10^{-7}$ nH	pH 8.1 = 0.79 $\times 10^{-8}$ nH
pH 6.2 = 0.63 $\times 10^{-6}$ nH	pH 7.2 = 0.63 $\times 10^{-7}$ nH	pH 8.2 = 0.63 $\times 10^{-8}$ nH
pH 6.3 = 0.5 $\times 10^{-6}$ nH	pH 7.3 = 0.5 $\times 10^{-7}$ nH	pH 8.3 = 0.5 $\times 10^{-8}$ nH
pH 6.4 = 0.4 $\times 10^{-6}$ nH	pH 7.4 = 0.4 $\times 10^{-7}$ nH	pH 8.4 = 0.4 $\times 10^{-8}$ nH
pH 6.5 = 0.316 $\times 10^{-6}$ nH	pH 7.5 = 0.316 $\times 10^{-7}$ nH	pH 8.5 = 0.316 $\times 10^{-8}$ nH
pH 6.6 = 0.25 $\times 10^{-6}$ nH	pH 7.6 = 0.25 $\times 10^{-7}$ nH	pH 8.6 = 0.25 $\times 10^{-8}$ nH
pH 6.7 = 0.20 $\times 10^{-6}$ nH	pH 7.7 = 0.20 $\times 10^{-7}$ nH	pH 8.7 = 0.20 $\times 10^{-8}$ nH
pH 6.8 = 0.158 $\times 10^{-6}$ nH	pH 7.8 = 0.158 $\times 10^{-7}$ nH	pH 8.8 = 0.158 $\times 10^{-8}$ nH
pH 6.9 = 0.125 $\times 10^{-6}$ nH	pH 7.9 = 0.125 $\times 10^{-7}$ nH	pH 8.9 = 0.125 $\times 10^{-8}$ nH

Careful determinations of the hydrogen-ion concentration of the sea water and of the body fluids of a wide range of animal forms have been made. Table 28 presents the facts as they are at present known and indicates that with very few exceptions the body fluids of all animals have a pH value slightly on the alkaline side of neutrality. The exceptions noted in the table may possibly be explained by the fact that there is secreted in certain of the blood cells a vanadium-containing compound which is in itself quite strongly acid.

TABLE 28.—TABLE SHOWING THE HYDROGEN-ION CONCENTRATION OF THE BODY FLUIDS OF A SERIES OF ANIMALS

SPECIES	pH
Alcyonium palmatum (Cœlenterate)	8.54
Alcyonium palmatum	8.43
Sipunculus nudus (worm)	7.79
Sipunculus nudus	7.25
Asterias glacialis (Echinoderm)	7.76
Astropecten	7.27
Sphærechinus granularis	7.60
Strongylocentrotus	7.63
Holothuria	7.67
Aplysia (Mollusc)	7.23
Eledone moschata	7.36
Octopus vulgaris	7.54
Maja (Crustacean)	7.45
Palinurus (Crustacean)	7.38
Homarus	7.62
Cyona (Ascidian)	6.56
Torpedo (Elasmobranch)	7.64
Strombus gigas (Mollusc)	7.5
Cassiopea	7.7
Pecten	6.5
Human blood (mammal)	7.4–7.49
Sea water	8.0–8.2

From what has been said it will appear that the circulating body fluid is not only a solution of relatively stable osmotic pressure and of definite composition as far as the ratios of metallic ions are concerned, but that it is also a solution of remarkably definite and constant alkalinity. This

means that the fluid must contain what are known as buffer substances. In this regard, again, the body fluid is like the sea water.

The Buffer Substances of the Blood and Carbon Dioxide Transportation.—The part which the blood plays as a carrier of carbon dioxide is one until recently very little understood. A number of ingenious suggestions have at various times been offered as to the nature of the compounds formed between carbon dioxide and substances in the blood, several of which have proved part truths.

The blood, like sea water, is a solution. In this case it is a solution of proteins and inorganic salts in water. Unlike the sea water the blood contains floating cells which themselves contain substances which may be a part of the chemical mechanism involved in the carrying of carbon dioxide as well as of oxygen. The difficulty in understanding the process comes from the complexity of the system involved in the transport, and our previous lack of ability to form a mental picture of the whole series of closely related chemical operations which have a part in the process. For much of the material contained in this account of buffer substances and their actions the author is indebted to the very able review by D. D. Van Slyke.

It has been claimed that the proteins of the blood (by which appears to be meant the proteins of the plasma) have the power to combine with the acids produced in the various chemical operations taking place in the body, and so serve to keep the body fluid from becoming too acid in reaction. As a matter of fact, the amount of protein present in the blood of even one of the higher animals is very small, making a solution of only 0.0023 molecular concentration. These proteins have very few NH_2 groups capable of combining with acids. It has been calculated that the proteins of the blood serum would be about equivalent to a 0.006 normal NH_3 solution and so could neutralize only a small amount of acid.

The acid which serves to keep up the hydrogen-ion concentration of the blood is in the nature of a waste product and comes from all the active tissues of the body. Carbon dioxide is produced by active cells generally; this carbon dioxide upon combining with water gives carbonic acid, which immediately ionizes, giving one free hydrogen ion.

Carbon dioxide may appear in the blood in any one of four ways: first, as anhydrous carbon dioxide; second, as carbonic acid (H_2CO_3); third, as a bicarbonate ($BHCO_3$), in which B indicates a monovalent base such as sodium or potassium; fourth, as carbonate (B_2CO_3).

It is probably true that the amount of anhydrous carbon dioxide is practically negligible, most if not all of the carbon dioxide combining at once with water to form carbonic acid. Of the carbonate and bicarbonate only the latter can exist in significant amounts in solutions containing as much carbonic acid as is found in the blood. Thus, practically, carbonic acid and bicarbonate are the forms in which carbon dioxide may

appear in the blood. If all of the carbon dioxide of the blood were present in the form of carbonic acid, the blood would be a thousand times more acid than it is; and if it were in the form of the carbonate it would be hundreds of times too alkaline. It is through the mechanism for maintaining the proper balance of the carbonic acid and the bicarbonate that the hydrogen-ion concentration of the blood is kept within its normal narrow limits, and that the carbon dioxide produced in different parts of the body is continually carried to the organs of elimination (lungs or gills).

Buffer Substances of the Blood.—Buffers are either weak acids or weak bases. They are characterized by the fact that they render solutions in which they are present able to receive additional limited amounts of either acid or alkali with much less changes of pH than would be caused by adding the same amounts of acid or alkali to water, or to a solution containing only salts, such as NaCl, which have no buffer value. All the important buffers of the blood, *viz.*, the bicarbonates, phosphates, and the proteinates of the alkali metals, are salts of weak acids. In each buffer part is present as free acid, part as the salt of a strong base, and the pH of the blood is determined by the relative proportions of buffer salts and free buffer acid respectively.

The alkali salts which constitute the buffers of the blood serve as reservoirs of alkali, a portion of which they give up to neutralized carbonic or any other acid that enters the blood. It is in this manner that the phosphates, the alkali salts of the plasma proteins, and the hæmoglobin of the blood act.

As an example, the case of sodium bicarbonate in solution may be cited. The solution of this salt is alkaline because the salt, being one of a weak acid with a strong base, is hydrolytically dissociated into H_2CO_3 and NaOH. The NaOH is much more dissociated than the carbonic acid and therefore yields an excess of OH ions. So, in a solution of $NaHCO_3$ containing CO_2 it may be stated that the former confers alkalinity and the latter acidity. The hydrogen-ion concentration of the solution as a whole would then depend upon the ratio of the one to the other. If the carbon dioxide increases without changing sodium bicarbonate, there will be an increased hydrogen-ion concentration. On the other hand, if there is a decrease of the carbon dioxide without a change in the sodium bicarbonate, there will be a lowering of the hydrogen-ion concentration.

The significance of the presence of the bicarbonate in the blood may be easily shown. In muscle, lactic acid is produced. Some of this lactic acid is diffused into the blood stream. Some of it may be further oxidized and converted into carbon dioxide. Such of the lactic acid which is diffused into the blood stream enters into combination with the sodium bicarbonate and carbon dioxide is given off. In either case the amount of carbonic acid in the blood is increased. Also, if the blood be subjected

to chemical analysis it will be found that there is an actual reduction in the amount of the bicarbonate present. This condition of reduced bicarbonate is spoken of as *acidosis*, not because there is an acid action in the blood, but because the reserve for overcoming the acidity is reduced.

Two general laws for buffer action have been stated by Henderson: First, the hydrogen-ion concentration of the buffer solution is proportional to the ratio free acid: free salt. Examples of such ratios in the blood are the ratio of carbonic acid to base bicarbonate, the ratio of dibasic phosphate (Na_2HPO_4) to monobasic phosphate (NaH_2PO_4), and the ratio of oxyhæmoglobin to the alkali salt of hæmoglobin.

Second, a given buffer is most efficient in maintaining constancy of pH when the ratio of the free acid to the basic salt is approximately 1.

The bicarbonates are much less efficient than the phosphates as buffers in the blood at the particular pH values which are normal for blood, but they occur much more abundantly in the blood cells than in the plasma. Neither the bicarbonates nor the phosphates have their maximum efficiency as buffers at the normal pH values of blood, but at pH 6.95, which appears to be the most acid point consistent with life in man, the phosphates come very nearly to their maximum efficiency. On the other hand, the organism has produced in the blood proteins, especially oxyhæmoglobin, buffers which are very nearly ideal, having in the case mentioned maximum efficiency at pH 7.7, which is very nearly that of the blood.

While the greater part of the phosphates of the blood is to be found in the corpuscles, a certain minimal amount occurs in the blood plasma. If the blood becomes more acid than normal, more and more of the acid phosphate ($NaHPO_4$) is formed. This passes through the glomerulus of the kidney into the filtrate. The phosphates are so dissociated in solution that NaOH and phosphoric acid (H_3PO_4) are present in the solution. Sodium salts are in constant demand by the living organism. On the other hand, phosphoric acid is wanted only in a small amount. The kidney tubules are supposed to absorb the sodium in the form of the bicarbonate, leaving the phosphoric acid behind in slight excess, so that the urine is slightly acid in reaction. If the phosphoric acid is present in greater excess than normal in the glomerular filtrate it will be left behind and escape with the urine while a normal fluid will be absorbed by the blood.

It is further found that the carbon-dioxide-carrying power of the oxygenated whole blood is about evenly divided between serum and cells, although the serum has only about one-third of the buffer content of the whole blood. It also appears that the cell buffers have the power of transferring some of their buffer effect to the more poorly buffered plasma, so that it, when in contact with the cells, becomes as efficient a carbon dioxide carrier as they. The cell buffers are chiefly the phosphates and

hæmoglobin. About 25 per cent of the total carrying power of the whole blood appears to depend upon the phosphates and something more than 70 per cent upon the hæmoglobin. Both of these substances do their work within the cell walls. Hæmoglobin is, of course, because of its colloidal character, unable to pass out of the cells, and apparently the phosphates do not do so.

It has been observed that when carbon dioxide is passed through blood there is an increase in the alkali titratable by methyl orange. This is presumably in the form of a bicarbonate. Such an increase could be the result of a diffusion of alkali out of the blood cells or the diffusion of an acid into them. Apparently, small amounts of HCl are formed by the action of carbonic acid and sodium chloride, and this diffuses into the cells, there to react with K_2HPO_4 which is present, in the formation of KH_2PO_4 plusK Cl. The following table from Van Slyke's *Review* indicates the possibilities of such diffusion:

TABLE 29

Plasma	Cell wall	Cell
1. $H_2CO_3 + NaCl \rightleftharpoons NaHCO_3 + HCl$	$\rightarrow HCl \rightarrow$	3. $HCl + K_2HPO_4 \rightleftharpoons KH_2PO + KCL$
2. H_2CO_3 + Na Protein $\rightleftharpoons NaHCO_3$ + H Protein		4. $HCl + KHbO \rightleftharpoons HHbO + KCl$
		5. $HCl + KHb \rightleftharpoons HHb + KCl$
	$\rightarrow H_2CO_3 \rightarrow$	6. $H_2CO_3 + K_2HPO_4 \rightleftharpoons KH_2PO_4 + KHCO_3$
		7. $H_2CO_3 + KHbO \rightleftharpoons HHbO + KHCO_3$
		8. $H_2CO_3 + KHb \rightleftharpoons HHb + KHCO_3$

Reaction 1 is supposed to account for from two-thirds to three-fourths of the total bicarbonate formed in the plasma of the whole blood by a rise in the carbonic acid such as to cause a pH change within the normal range. The removal of the HCl to the cells makes it possible for the reaction to proceed to a measurable degree.

Reaction 2 accounts for from one-third to one-fourth of the alkali bicarbonate formed in the plasma of the whole blood when the carbonic acid is increased within physiological limits.

The amount of bicarbonate formed in the cells in reactions 6, 7, and 8 is about equal to that formed in the plasma in reactions 1 and 2. And, presumably, in the HCl shift from plasma to cell, from two-thirds to three-fourths as much Cl is taken into the cells as bicarbonate is formed in them. This comes from the fact that reactions 3, 4, and 5 are equivalent to about two-thirds to three-fourths of reactions 6, 7, and 8 combined.

The division between buffer effects of phosphates and hæmoglobin is an uncertain one, but probably the hæmoglobin has about three times the buffer value of the phosphates. It is also found that the buffer values of oxyhæmoglobin and reduced hæmoglobin are not the same. Reduction of the blood allows it to take up 5.5 volumes per cent of carbon dioxide without increasing its acidity, since it is taken up in the form of the bicarbonate and thus makes a total carbon dioxide capacity of 99 cubic centimeters per 100 cubic centimeters of blood.

Carbon Dioxide in the Blood of Marine Invertebrates.—Parsons and Parsons, following the lead of Winterstein, have made an investigation of the carbon dioxide contents of the bloods of some of the marine invertebrates. Winterstein had attempted to measure the gas content of the body fluids of the invertebrates. Later Collip made a determination of the alkali reserve, or total alkali available in the body fluids of some of the invertebrates. Parsons and Parsons have attempted to plot out the complete carbon dioxide dissociation curves of the bloods of some of the larger and more common invertebrates found in the Mediterranean Sea at Naples. They found that the plasma of the blood of Ascidia contains decidedly less carbon dioxide than is dissolved in the sea water at the temperatures studied. The blood of the Mollusc Aplysia is not appreciably different from the sea water in its carbon dioxide content. The bloods of Octopus and of Palinurus have a much greater carbon dioxide content than the sea water and appear to be especially adapted for carrying this gas. The amount of carbon dioxide taken up by the bloods of Maia, Palinurus, or Octopus at any given tension of the gas is, in general, about two or three times that taken up by the sea water under the same conditions.

The authors show that at low carbon dioxide tensions the dissociation curves are steeper than at higher tensions, and that the animals make use of that portion of the curve in which there is involved less change in the reaction of the blood through the taking up of carbon dioxide. The pH of the blood of invertebrates appears about the same as that of the vertebrates (except in the Tunicates). The blood of the invertebrates contains more sodium bicarbonate than does the sea water. The blood proteins (hæmocyanin and others) act as weak acids and expel carbon dioxide from the blood at the low tensions which include the physiological range The acid-neutralizing power of the blood of the invertebrate is roughly proportional to its protein content. In the invertebrate blood the proteins occur as sodium proteinates, which are capable of giving up sodium for the transport of carbon dioxide in the form of sodium bicarbonate.

CHAPTER XII

THE COAGULATION OF THE BLOOD AND PHAGOCYTOSIS

One of the most interesting and significant phenomena associated with the internal medium of the living body is the manner in which insurance against loss of fluid is attained in various forms. In this regard there seems to have been a very definite evolution. The examples cited will give sufficient evidence that this is true, although they may not clearly indicate all of the steps in the evolutionary course.

By coagulation is meant the change, from a liquid to a gelatinous condition, which takes place in the blood when it is shed from the blood vessels. In the very lowest forms in which a definite circulating fluid is found there is certainly no evidence of any provision in the composition of the blood which might lead to the formation of a blood clot. In the higher forms such a provision is made in one or another form.

The perivisceral fluid of the sea urchin is a fluid of a specific gravity similar to that of sea water. In this fluid are numerous amœboid corpuscles which, when the blood is shed from the body, tend to collect and to send out long pseudopods which become entangled, finally merging the cells into a homogeneous mass. This mass then sends out long pseudopods and entangles all the corpuscles within reach and draws them into itself in such a way that a large plasmodium is formed which soon differentiates into a transparent ectoplasm and a granular entoplasm. The coagulation here seems entirely a function of the cells and not of the plasma at all.

In the Mollusc *Cardium norwegicum*, Drew observed the agglutination of the corpuscles due to contact with foreign bodies. Apparently, when a corpuscle has adhered closely to some surface, it develops the power of adhering to any other corpuscle with which it may come into contact. This condition does not exist in freshly drawn blood. Two corpuscles that have adhered may separate; when this occurs a very fine strand (sometimes invisible) is left connecting the two cells. This strand may serve as an entanglement to other corpuscles. The corpuscles at either end of the strand may travel along the strand and appear to become merged in it. The strand contracts upon standing and with such force that two original masses of agglutinated corpuscles may finally become merged into one. The agglutinated corpuscles appear more or less structureless and very much resemble a mass of fibrin.

In the blood of Limulus, the appearance of fibrin as a definite component of the blood has for the first time been mentioned. It is to be

noted, however, that in this case the fibrin is not a component of the plasma of the blood, but of the corpuscles, and as such is present in relatively small amount, due to the comparatively small amount of the corpuscular material of the blood.

Howell gave, some years ago, the first account of the coagulation of the blood of Limulus, which, while not so detailed as the explanations and accounts at present available, certainly is a record of careful and accurate observation. The corpuscles, after the blood has escaped from the blood vessels, almost immediately send out processes which unite with other corpuscles or with the pseudopods coming from them. By the shortening of these filaments the corpuscles are brought together into a more or less compact mass. Howell states that in its most important properties of solubility the fibrin of Limulus resembles that of the ordinary mammalian blood, although it is composed directly of corpuscles.

Leo Loeb and Alsberg and Clark have recently studied very carefully the processes of coagulation of the blood of Limulus. From the work of these authors it appears that Howell's observations were in the main correct. When Limulus blood is shed there is a definite agglutination of the corpuscles, and the same effect may be produced if heat-coagulated proteins are introduced into the body of a Limulus. The blood cells gather about any solid foreign body and form a thrombus-like mass. The coagulation seems, then, to consist of an agglutination of the blood cells and of the cell protoplasm when it has left the cell body. Fibrin or fibrinogen does not seem to be present at all in the plasma of Limulus blood, nor in the blood of any invertebrate lower in the animal scale than the Crustacea. The insoluble material which forms the substratum of the clot is termed *cell fibrin*. There is no evidence whatever that it is in any way allied with the fibrin found in the blood of the higher animals. It is an albuminoid substance, and related to the glutin or elastin groups, probably the former. Such a substance has not often been found in an invertebrate.

The bloods of the Crustacea show a distinct evolutionary advance in the matter of the method of conservation of body fluids. Tait has distinguished three modes of coagulation:

1. Simple agglutination of the blood corpuscles without any subsequent jellying of the plasma. This is probably the most primitive and essential device in both invertebrates and vertebrates for procuring arrest of hæmorrhage from a wound. This method appears in *Cancer pagurus*, Maja, Squinado, the spider crab Inachusdorynchus, Macropodia, Rostratus, etc.

2. Agglutination of corpuscles with subsequent general jellying of the whole mass. This form of coagulation appears in the genera Carcinus, Palæmon, Portunus, and Homarus.

3. Jellying of the plasma in two successive stages, the preliminary cell agglutination being relatively insignificant. The first plasma coagulation consists of localized (primitively globular) clots which occur around or in immediate relation to special blood corpuscles originally discovered by Hardy (1892) and called by him *explosive corpuscles*. At a later stage a second jellying takes place which, this time, involves the whole of the remaining plasma. This is the most advanced coagulative condition found among the Crustacea and occurs especially in Palinurus and Astacus and among the Isopoda. It also occurs in the Amphipod *Gammarus locusta*, but is not found in some others of the same genus.

The third form or type of coagulation as found in Astacus and others of the higher Crustacea is of such interest as to warrant a somewhat more extended account. The phenomena accompanying coagulation are very difficult to follow without the aid of stains. When a fresh drop of blood of the crayfish Astacus is placed upon a glass slide, there occurs within a few seconds a very complete cytolysis of the explosive cells. If, then, there be allowed to run under the cover glass of the preparation a drop of iodine solution, it will be seen that the nuclei of the explosive cells are embedded in strands of fibrin which run in the direction taken by the iodine current. If left for a longer time before the mixing of the fixing and staining fluid, it will be seen that each of the nuclei of the explosive cells is at the center of a circular island of coagulum. Whether the fibrin appears in the form of threads or of islands, it is supposed in each case that the fibrin owes its origin to the escape from the explosive corpuscles of a thrombin, which then affects some coagulable substance in the plasma. It evidently requires some little time for the soluble material or thrombin of the corpuscle to diffuse from the cytolyzing cell. If in the meantime a current be set up in the plasma, the thrombin will be carried away in stream lines. If the plasma be undisturbed by currents, the substance given off by the cell will diffuse almost equally in all directions and give origin to the so-called island. The coagulum has no tendency to spread or to enlarge its boundaries. This fact seems to be an argument for the idea that there has been a using up of material derived from the cell in the formation of the clot. The thrombin cannot, therefore, in this case be considered as a ferment in the true sense of the term. It is probably correct to say that a fixed quantity of the thrombin is incapable of coagulating an indefinite amount of the plasma. Under special conditions the fibrin threads may be found to reach for considerable distances from the cells (at least 1½ millimeters).

Some 20 to 30 minutes after the first coagulation of the blood, the second or thigmocyte coagulation occurs. The cytolysis of the thigmocytes is somewhat delayed, but when it does occur there is thrown into the plasma a new supply of thrombin which acts in the same way as that first given off by the explosive cells. There is formed about the remains

of the thigmocyte a coagulum of the same character as that first formed about the remains of the explosive cells.

No production of thrombin by the amœbocytes has been determined, nor has anything been seen to indicate a causal relation between them and the formation of a clot.

The cause of the liberation of the thrombin by the cells is held to be due to the contact of the blood with a non-greasy material. This sets up a cytolysis of the more sensitive elements of the blood. When the blood is kept in liquid paraffin after removal from the blood vessels, these cells remain intact and no coagulation occurs.

Zoölogical Significance.—The various categories into which the Crustacea fall according to the type of blood coagulation do not coincide with zoölogical groupings. Among the Decapods all three kinds are found. It is very doubtful if, as yet, sufficient information has been procured to justify using blood coagulation as a means of deciding relationships among the Crustacea. In all of the invertebrates except the Arthropods the only form of coagulation known to occur is an agglutination of the corpuscles. There is no jellying of the plasma. In Arthropods alone is there a true jellying or solidification of fibrinogen normally present in the plasma. Among the Crustacea are all possible gradations of the process from complete absence of jelly, as in spider crabs, to the occurrence of a very firm jelly coagulation, as found in the lobster and rock lobster. It is also found that the jellying process, when present in what is possibly its most developed state, is associated with the presence of corpuscles having special physiological attributes.

Fig. 18.—Red corpuscles of frog blood photographed by dark-field illumination.

Frog's Blood.—The blood of the frog (Fig. 18) contains, in addition to the usual array of red and white corpuscles, certain specialized cells known, from their shape, as *spindle cells*. These are present normally in the blood to the number of about 16,000 per cubic millimeter. (There are about 7000 leucocytes per cubic millimeter in the same blood.) These spindle cells are very unstable and are not found in frog blood which has been shed for any length of time, unless special precautions are taken. The spindle cells may be rendered less liable to disintegration upon extravasation if some anticoagulant, such as sodium fluoride, is added to the blood. This can be done by injecting into the dorsal lymph sac 1 cubic

centimeter of a 1.2 per cent solution of sodium fluoride in 0.6 per cent sodium chloride. The frog is then placed in a bath of chloroform water, and after 10 minutes the animal is removed and the blood collected for examination. The spindle cells are elongated, one end usually tapering and ending in a pointed tip, while the other end is shorter and more blunt. The long axis of the cell is not always straight. The cell is flattened from side to side, and one edge is likely to be thicker than the other. The nucleus of the cell is a long shadowy structure near the blunt end of the cell. The cytoplasm of the cell is hyaline or finely granular, and stains irregularly and never deeply. Sometimes the spindle cells become more elongated, having the nucleus at the broad end of the cell and the other end reaching out in a long tail, two to three times the length of the nucleus, and bearing at its end a small knob.

Coagulation of Frog's Blood.—The coagulation of the frog's blood occurs in two phases. When this fact was first observed, it was thought that this process is comparable with the process of coagulation which occurs among the Crustacea. The resemblance has been found to be, however, only a superficial one.

The first stage of coagulation is characterized by the appearance in the blood of minute, disc-like islands, here and there in the blood plasma. These islands settle to the bottom of the container, but do not adhere to each other or to the dish. The smallest of them are invisible to the unaided eye, but the largest are as large as the head of a pin. This first stage of coagulation is found not to be made up of areas of localized coagulation about the remains of one or more spindle cells. Each island consists of a central mass of red cells, surrounded by a zone of cells, most of which are spindles. The island is built up by an original clumping of five or six spindle cells. Gradually the group increases in size through the addition of more spindle cells. The cells occupying the center of the mass lose their spindle form, becoming shorter, broader, and more or less rectangular in outline. The cytolysis which is the characteristic feature of clot formation among the Crustacea does not occur.

After these island masses have become well established—a process which may involve considerable time—a jellying of the whole mass of the plasma soon follows. The details of this portion of the process of coagulation have not been fully worked out.

Coagulation of Mammalian Blood.—When blood from the blood vessels of one of the higher animals is shed into a glass dish, it changes within a few minutes from a liquid condition to a more viscous liquid and then to a jelly-like form. The rapidity with which the process takes place and the degree of consistency of the jell produced vary greatly in different species of animals, and sometimes even with animals of the same species. Frequently the blood in a beaker will, within a few minutes, become so thick that the container can be inverted without any loss of

blood. The clot or coagulum sets more and more firmly, and at the same time begins to contract. As it contracts it squeezes out from itself a variable amount of a clear yellowish liquid, called blood serum. The essential portion of the clot is the fibrin (see Fig. 19). This is a stringy, insoluble protein, not found in normal blood. It can be shown that this substance can be and is formed from a soluble substance of the blood plasma, the fibrinogen. In ordinary microscopical preparations the fibrin appears to be deposited in the form of longer or shorter threads, frequently leading from one leucocyte or platelet to another, and forming a network in which the red corpuscles or erythrocytes are caught. Upon examining coagulating blood upon a slide by means of the ultra-microscope,

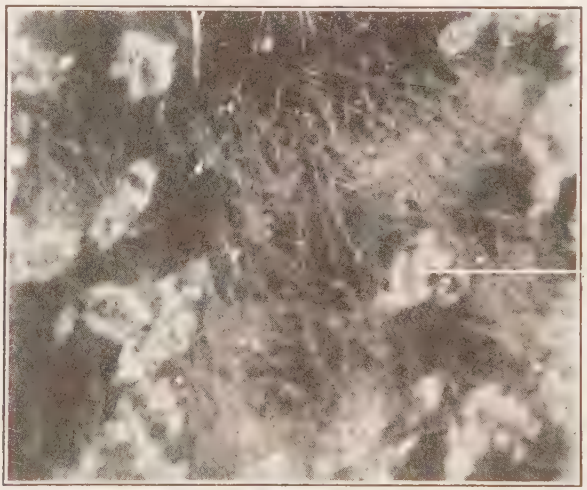


FIG. 19.—Photograph by dark-field illumination of coagulum of human blood. The network consists of fibrin filaments reaching across spaces in the plasma between masses of red corpuscles. At *L* is shown a large polymorphonuclear leucocyte.

it is possible to see, in addition to the threads of fibrin, small island-like masses of coagulum developing about the remains of some of the colorless corpuscles. Dr. Howell has pointed out that the apparent threads are in reality very fine needle-shaped crystals which ultimately form a very dense meshwork. He calls attention to the fact that this form of precipitation of a hydrogel from a hydrosol is unique as far as our knowledge goes.

Many suggestions have been made as to the nature of the phenomena involved in the coagulation of the blood of the higher forms, but none has received unanimous support. The phenomenon of coagulation is primarily a change of one of the colloidal constituents of the plasma from the sol to the gel condition.

The blood plasma contains, among other things, a soluble protein known as fibrinogen. According to Howell, the clotting of mammalian blood takes place in two stages: (1) the formation of thrombin from antecedent substance existing in the body; and (2) the action of the thrombin on fibrinogen whereby fibrin is formed and deposited as a gel or clot. Prothrombin, which is normally present in the blood plasma, is supposed to be converted into thrombin by the action of calcium alone. In the circulating blood the activation of the thrombogen or prothrombin does not take place because of the presence of an inhibiting substance, antithrombin, in sufficient amounts to prevent the reaction. When blood is shed, the tissue cells or the blood platelets furnish a substance, cephalin protein, which neutralizes the antithrombin and thus permits the calcium to react with the prothrombin to form thrombin, which in turn reacts with fibrinogen to form fibrin.

The theories of Morawitz and of Fuld and Spiro differ in one very important point from that of Howell. They assume the presence of calcium and of thrombogen in the blood plasma. They also contend for the existence of a substance, thrombokinase, which is produced by tissue cells and by some of the cellular elements of the blood, particularly the blood platelets. According to this theory the reactions are as follows:

Cellular elements give up thrombokinase.

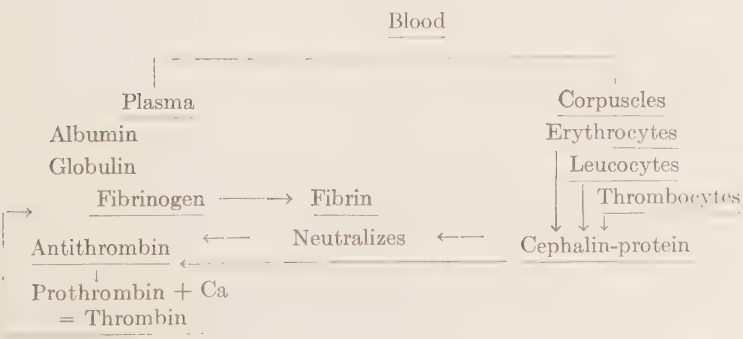
Thrombokinase plus calcium plus thrombogen = thrombin.

Thrombin plus fibrinogen = fibrin.

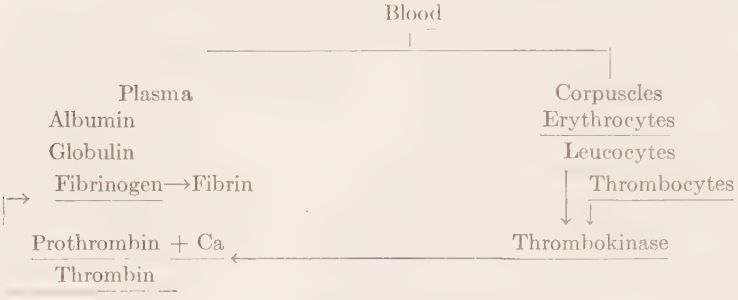
It is to be noted that the central action in the theory as stated is one of an enzyme type induced by the kinase. Authorities are not at all agreed that such a thing as an active ferment exists in the blood. In case the thrombokinase is not a substance of the ferment type, the theory falls.

The following diagrams summarize in brief form the theories of Howell and of Fuld and Spiro as to the manner in which the coagulation takes place.

HOWELL'S THEORY OF COAGULATION



FULD AND SPIRO'S THEORY OF COAGULATION



PHAGOCYTOSIS

One of the most important functions served by blood cells is the ingestion of foreign particles. This habit is apparently a direct inheritance from ancestral forms of cells and would naturally be expected to occur in cells so little differentiated as white corpuscles. Among the higher animals this property has become highly specialized as a means of protecting the organism against the attacks of disease germs.

When foreign particles are ingested by blood cells, several possible fates lie before them:

1. If they are particles of dead material capable of being digested, the cells may secrete some digestive fluid, as is done by protozoa, a food vacuole may be formed, and the particle may gradually be dissolved.

2. If the particles are living, the digestive juices of the phagocyte may kill them, in which case digestion soon follows.

3. If the particles are living and prove to be highly resistant, they may not be digested but may grow and even multiply within the phagocyte, finally to escape within the cell and become distributed throughout the organism.

4. The particle may be indigestible. In this case the cell may carry it about for a time but will finally discharge its burden at some point in the organism. This is one of the methods utilized by some of the lower animals for taking care of excretory matters and in certain cases for the transportation of food matters. It is also used to some extent in the higher animals for disposing of wastes and of foreign materials which collect on exposed epithelial surfaces.

The work of the phagocytes is not only largely chemical in character but the cells are to a large degree controlled in their activities by chemical agents. The collection of phagocytic cells at foci of infection is a clear case of chemotropism. In this case the stimulating substance is some substance produced as the result of the metabolic activities of invading disease germs or some substance formed during the breakdown of tissue cells. Fenn has shown that the adhesiveness of leucocytes to glass may be modified by changing the hydrogen-ion concentration of the liquid.

Immediately on contact with glass, leucocytes are most adhesive at pH 8 or above. Agglutination of the corpuscles increases from pH 8 to pH 6. The optimum condition for phagocytosis by creeping leucocytes occurs at pH 7. The optimum pH for phagocytosis of quartz particles in suspension is at about pH 6.7 and increases with acidity, whereas the adhesiveness of leucocytes to glass decreases with an increase of acidity. In many cases the taking up of foreign organic particles is conditioned by the existence in the blood of an opsonin, a substance which apparently renders the particle attractive to leucocytes. If all the instances of phagocytosis are similar in mechanism, one may formulate the following relationships between opsonins and agglutinins: Both are substances (or conditions) that produce adhesion of suspended particles; an agglutinin causes two elements of the same variety to adhere, while an opsonin produces adhesion between two different elements, one of which, the phagocyte, is skin-less and therefore penetrable by the other. From this point of view the agglutination of skinless cells, like thrombocytes, is a mutual phagocytosis.

While the white blood corpuscles are the most conspicuous examples of cells which act as phagocytes, there are many other cells which also exhibit phagocytic power. Among these are the cells of the spleen pulp, of lymph glands, nerve cells, neuroglia cells, and certain endothelial cells.

It has already been pointed out that Tait found in the blood of Ligia at least three types of cells—the explosive cells, hyaline thigmocytes, and granular thigmocytes. The two latter groups show marked thigmotactic properties on glass. The granular thigmocytes are distinctly amœboid in character; the hyaline thigmocytes and explosive cells expand upon a clean glass slide into a thin sheet and are not able then to retract their protoplasm. The phenomenon observed in this spreading appears in no way different from that seen in the spreading of water over a surface to which it adheres. It is a purely physical phenomenon.

Tait then found that in the Mollusc Ligia the hyaline and granular thigmocytes are both phagocytic. In the crayfish it also appeared that the hyaline and granular thigmocytes can ingest particles of carmine, India ink, glass, etc., and that the hyaline cells are the more active in this regard. Tait calls attention to the fact that, so far as behavior on glass is concerned, the mammalian platelets behave as do the hyaline thigmocytes. The strong adhesion manifested by the amphibian spindle cells and crustacean thigmocytes toward glass naturally suggests that the process may be a physical one. Accidental contact of particles to the surface of the cell would lead to adhesion. The same causes which lead to the spreading of the cell substance on the glass may also lead to the ultimate engulfing of the particle by the cell substance. Capacity for amœboid movement is not an essential property of the phagocytic cell, for the hyaline thigmocytes are not amœboid.

Among the blood cells of the higher animals which act as phagocytes, the most important are the microphages, the polymorphonuclear and eosinophil leucocytes and the macrophages, the large hyaline blood cells. To these should be added cells of the spleen pulp, endothelial cells, and neuroglia cells. The microphages appear to be most concerned in combating acute infections, while the macrophages have to deal with chronic infections.

It is interesting to note that there is a great deal of difference in the phagocytic activity of corpuscles with reference to such substances as carbon and quartz particles, the former being ingested much more rapidly than the latter. The temperature coefficients for the phagocytosis of these inert particles increase rapidly below 30°.

Fenn has made an elaborate investigation of the phagocytosis of solid particles. His fundamental concept was that the taking up of the foreign particles by the white blood cells can be explained upon purely physical grounds, and the number of particles ingested by the cells follows definite mathematical laws. Previous investigators had taken the position that the factor which determines how many particles will be taken up by the cells is the number of actual collisions which take place between phagocytic cells and suspended particles. Fenn contends that it is the *chance of collision* which is the determining factor. In determining the actual rate of phagocytosis, it was found best from time to time to remove samples of the suspension to a counting chamber and to make determinations of the numbers of the particles not ingested, rather than to attempt to determine the number that had been ingested. The suspensions for this work are made up with a great deal of care to have the particles of as nearly uniform size as possible. The chances of collision between the cells and the particles are determined mathematically, and vary with the speed at which the liquid is stirred, the velocity of movement of the leucocytes and their size, and the size of the particles. The density and viscosity of the liquid must also be taken into account. The measure of phagocytosis is the percentage of the collisions occurring between cells and particles which result in ingestion. In any one experiment the number of cells is constant, and the relative number of collisions is proportional to the number of particles.

Fenn has made a study of the effects of temperature upon the phagocytic reaction. In this connection he has made an analysis of data given by Madsen and Watabaki and shows that there are probably three different reactions involved in the phagocytosis of bacteria. These are:

1. A preparatory reaction represented by the latent period during which the leucocytes originally inactive are rendered active.

2. A lethal reaction which injures the leucocytes so that they are again inactive.

3. The phagocyte reaction proper.

These reactions may be shown diagrammatically as follows:
Let C be a blood corpuscle, CB a corpuscle containing bacteria.

$$C_{\text{inactive}} \xrightarrow[\text{(1) } Q_{10} = 2.3 - 2.6]{\text{Preparatory reaction}} C_{\text{active}} \xrightarrow[\text{(2) } Q_{10} = 1.3 - 1.7]{\text{Lethal reaction}} C_{\text{inactive}}$$

$$C_{\text{active}} + \text{Bacteria} \xrightarrow[\text{(3) } Q_{10} = 2.0]{\text{Phagocytic reaction}} CB$$

Fenn finds that the rate of phagocytosis is a logarithmic function of temperature from 0 to 33° and that the valve $Q_{10} = 2.0$ is applicable over the whole range when the ingestion of bacteria is concerned.

Fenn discusses the part which surface tension may play in the taking up of solid particles by the cells. He shows mathematically that:

1. If a cell spreads to infinity on a flat surface of G, it will completely ingest a particle of G.

2. If a cell does not stick to a flat surface of G, a small particle of G will not stick to the cell.

3. If a cell is partially spread out on a flat surface of G, a particle of G will be partially ingested.

The relative surface energy has been calculated at various stages in the ingestion of a solid particle by a fluid cell with a diameter four times as large as that of the particle, and it is shown that no particle will be ingested until the surface tensions are such that the cell would spread to infinity on a flat surface of the same substance. The adhesiveness of blood cells to solid substances is shown to be a pure surface-tension phenomenon, but in most reactions between living cells and solid bodies the fluidity of the protoplasm is also a factor of prime importance. The frequent occurrence of adhesiveness as a property of cells in contact with solid bodies is due to the fact that by so adhering the surface area of the cell not in contact with the solid is decreased.

CHAPTER XIII

CIRCULATORY MECHANISMS

Attention has already been directed to the fact that a circulating medium is an absolute necessity in the multicellular animals in order to provide that each cell shall receive its due share of oxygen, and food supplies, and that its wastes shall be removed. The earliest and lowest of the multicellular animals had their bodies more or less opened to access by the medium in which the animals lived. Such is the condition existing in the bodies of the sponges and Cœlenterates of today. As the animal bodies slowly increased somewhat in complexity, and as the internal body fluid came to be more or less shut off from communication with the outer medium, there came to be a need on the part of the organism for several different mechanisms. One of these is a mechanism for the secretion of the internal fluid. Another is a mechanism for the production of the various sorts of cells which are to become a part of the internal fluid. Still another is a mechanism for bringing about a circulation of the fluid, and still another is a mechanism for regulating the flow of the fluid through the animal body.

The mechanism for the secretion of the internal fluid is in part at least the epithelium of the alimentary tract. The epithelium of gill structures may also be involved to a certain extent in water-living animals. Associated with the digestive epithelium there is also the epithelium of the various forms of excretory organs which have to do with the regulation of the water content of the body, the alimentary organs and the excretory organs working against each to a certain extent.

In the lowest of the metazoa there is no special organ set aside for the production of blood cells. The cells which appear in the blood or other body fluid are simple mesoblastic cells which have become broken away from the walls of the vessels in which the body fluid circulates. Higher up in the animal kingdom there are set aside special glandular structures whose business it is to form and pass on into the blood stream the various kinds of cells to act as scavangers, etc.

Circulation among Protozoa.—It does not seem possible to say that in the protozoa there is any such thing as a circulatory mechanism, and yet there is a distinct and definite movement of the protoplasm in a more or less well-defined circuit. It is not known how this flow of the protoplasm is maintained. The movement of the protoplasm in animal cells is no doubt very similar to that of plant cells, which has been described

many times as streaming movements. This movement of the protoplasm serves to bring food vacuoles, with their content of food material in the process of digestion, into contact with all parts of the protoplasm. As the vacuole moves along the various products of digestion are given off from it, so that all parts of the protoplasm have the opportunity to pick up such substances as may be needed for their use.

Circulation among Sponges.—Among the sponges the sea water is, of course, the circulating fluid. There is nothing in the nature of a blood system, but there exist channels through which the water is passed freely, being kept in slow progressive movement by means of certain flagellated cells which line the radial water channels. That the flagella move with a considerable force is seen from the fact determined by Parker that there is developed in the cloacal chamber a pressure of from 3.5 to 4 millimeters of water in the case of Stylotella, and somewhat less in the other forms which he studied. The amount of water which could be passed through the sponge body in the course of a day is very considerable. Parker determined for Spinosella the volume of water and the rate at which it flows through the osculum. The rate in the particular case studied was nearly 4 millimeters per second and the amount of water discharged was about 0.9 cubic centimeter per second. At this rate the amount of water discharged in the course of a day would be some 78 liters. Parker calls attention to the fact that a specimen of this sponge studied may have as many as twenty oscula. Such a colony of sponge might pass through its body in the course of a day as much as 1575 liters of water.

Factors Involved in the Evolution of Vascular Systems.—Passing from a consideration of the conditions existing among the sponges to those which exist in higher forms of animals, a little speculation as to how the latter have come to be developed is only natural. As the bodies of the multicellular animals began to be more or less closed to the outer medium and there came to be an internal medium as distinguished from the outer, there arose a tendency on the part of the animals to form within themselves more or less definite channels along which the internal fluid might run. Whether these channels were laid down as a form of response to a demand upon the part of the organism of whether the animal made use of chance variations in its internal structure for directing the flow of the liquid medium here and there in the body is a question which is not yet settled, and which requires no particular concern at present. Dahlgren and Kepner in "Principles of Animal Histology" have suggested certain possibilities as to the formation of distributing channels throughout the animal body. In the early stages of differentiation, these channels in the body substance had no other boundaries than the mesodermal cells among which they had come to lie. Some of the mesodermal cells would even become detached from the body mass and float freely in the liquid. Such cells might become blood corpuscles or might be used in

forming definite walls of the vascular passages. An early type of differentiation was that which resulted in the formation of one or more long tubules or cavities running lengthwise of the body and from which branches might extend into any existing appendages. In such chambers the liquid was moved about by the general muscular movements of the body, but there was no definite circulation in any fixed direction.

A little later, as spaces with definite boundaries came to be developed in the animal body, there was a prophecy of lymph spaces and of blood capillaries. As these vessels became distinctly marked off, there came to be developed along the walls contractile fibers which could impart a rhythmic activity to the wall and so aid in the propulsion of the fluid contained within the tubule. The lymph capillary may therefore be thought of as the earliest type of vasculo-motor apparatus. Such lymph spaces, irregular in form, and branching and anastomosing in an irregular manner would penetrate to all parts of the organism, and so make possible the direct nourishment of all somatic cells.

The blood capillaries, small tubes made up of endothelial cells, anastomosing freely with each other, may have been next in appearance. The endothelial cells forming the walls of the blood capillaries may themselves have been contractile and so have served in a measure to assist in regulating the flow of the body fluid to this or that portion of the body. Early in the history of these vessels muscle fibers were laid upon the endothelial cells. When this was accomplished, the tubules were in condition more effectively to regulate the flow of blood through the body. This regulation could not, however, be very satisfactory until the muscle fibers had nervous connections. It will be pointed out in another connection that the nervous mechanisms of the lower organisms take the form of nerve nets, consisting of very primitive nerve cells, arranged more or less in the form of a network, and provided with processes which pass to other similar cells and to muscle cells. This was the type of structure which very early became associated with the muscle cells of the capillary walls. Such a network serves two prime functions: It acts, first, as a receptor of impulses, and, second, as a structure for giving rise to motor impulses. When the smaller blood tubes became equipped with muscle fibers under nervous control there existed a mechanism for controlling the caliber of the blood vessels under various conditions.

Following the equipment of the smaller blood vessels with muscle cells and nervous control, and probably coincident with an increase in the mass of the whole organism, there came a spreading of muscle and nervous tissue over one or more longitudinal tubes, and the taking on of more or less regular rhythmic contractions which would have the effect of forcing the blood along definite pathways. Such an arrangement is seen in the well-developed dorsal blood vessel of the earthworm.

The later development of the vascular apparatus was coincident with certain physiological demands. It is almost impossible to tell what is cause and what is effect. The development of the metazoan forms involves increase in size, that is, in body mass. Every increase of body mass, that is, increase in the number of cells included within a single living organism, must include a corresponding increase in the system involved in furnishing oxygen ond foodstuffs to the cells. There must be a correlation between active body mass and available oxygen and food supply (oxygen and food supply for the cells). As the mass of the body increases, there may be simply an increase in the amount of fluid within the body. Such an increase involves increase in the bulk of the organism, and in the case of a massive animal would not be effective unless some means of keeping the body fluid in motion were also developed, and unless

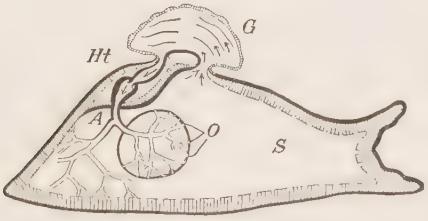


FIG. 20.—Diagram of the circulation of the snail *Aplysia*. *Ht*, heart; *G*, gill; *A*, artery; *O*, ending of the arteries and the great sinus; *S*, sinus.

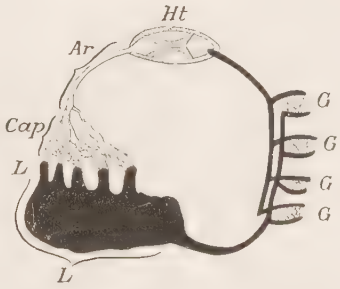


FIG. 21.—Diagram of the circulation of *Palæmon Squilla*. *Ht*, heart; *Ar*, artery; *Cap*, capillary; *L*, blood lacuna; *G*, gill.

the fluid came into close association with all the cells of the body. If some means can be developed for keeping the body fluid in movement, a smaller amount of it will suffice than if it is allowed to be stagnant. The correlations may be expressed in another way as being the ratio of:

Active body mass to body fluid mass.
Active body mass to width of capillary bed = intricacy of capillary network.
Active body mass to rate of blood flow } involving development of motor apparatus
Active body mass to blood pressure } and peripheral control.

The active body mass of the organism depends not only on the number of cells composing the organism but on the rate at which those cells are carrying on their metabolic processes.

It is impossible here to describe in detail the circulatory systems of the different animal groups. It must be assumed that the reader is familiar with the main types of circulatory apparatus as they appear among both invertebrates and vertebrates.

For convenience a few figures are introduced here which may call attention to the definite evolution which has occurred in the development of the vascular apparatus of the higher animals.

In Fig. 20 there is represented diagrammatically the circulatory system of the snail Aplysia. In this form there is an incomplete tubular system such as is characteristic of the Mollusca. The heart consists of two chambers, auricle and ventricle. The ventricle propels the blood through the arterial system, the fine branches of which bring the blood into contact with the most remote parts of the body. At the ends of the smaller arteries the blood enters the great sinus *S*, from which it finally passes through the aerating organ *G*, and thence back to the heart.

In Fig. 21 there is shown diagrammatically the circulatory system of an Arthropod *Palæmon squilla*. Here again is seen an incomplete tubular system. In this case the main artery ends in a system of capillaries. These terminate in lacunar spaces which finally carry the blood on to the gills, where it is aerated, and from which it passes directly to the heart.

Fig. 22.—Diagram illustrating the heart and main arteries of the circulatory system of the lobster. No attempt is made to indicate sinuses or bronchial vessels. *Ant.Ar.*, antennary artery; *Oph.Ar.*, ophthalmic artery; *Hep.Ar.*, hepatic artery; *Ht.*, heart; *Abd.Ar.*, abdominal artery; *st.Ar.*, sternal artery.

Figure 22 shows the general plan of arrangement of the arterial system of the lobster. Here again is found a system adapted to the needs of an animal which does not make a great demand for oxygen.

Vascular Apparatus of Bee.—The vascular system of the bee (see Fig. 23), as of other insects, is exceedingly incomplete. There is a tubular heart extending through several segments, and extending anteriorly from it is a long slender artery or aorta. The heart lies in a pericardial sinus formed by the shutting off of a part of the body cavity by a transverse sheet of connective muscle tissue known as the dorsal diaphragm. On the ventral side of the body a similar sheet known as the ventral diaphragm forms a similar ventral sinus. The heart is a segmental structure, and has several openings or ostia along its sides. These allow blood to enter the heart from the pericardial sinus. The blood which enters the heart through the ostia is prevented from escaping through them, when the heart contracts, by osteal lips which extend inward and forward from the openings, and which act as valves. Pressure against these valves during the contractions of the heart closes them and prevents escape of blood; at the same time the contraction of the heart brings opposing valves together

and so serves to prevent a backward flow of the blood. Concerning the circulation of blood in the bee Snodgrass says:

The circulation of the body liquid and the course it follows through the abdomen may be demonstrated easily by the following method, since the heart

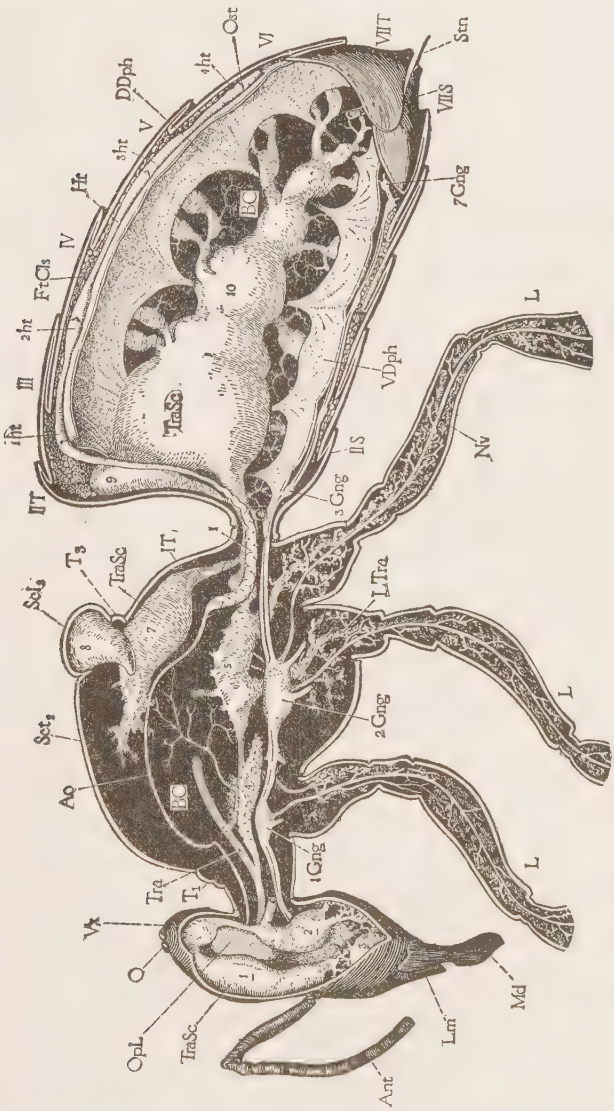


FIG. 23.—Vascular apparatus of bee. (*Snodgrass, Fig. 72. By permission of the McGraw-Hill Book Company, Inc.*)

and the diaphragms continue to pulsate for some time after the other body muscles are paralyzed by an anæsthetic or the body dismembered. Pin an asphyxiated bee to a block of cork or paraffin and remove the top of the abdomen by making a horizontal incision all around it with a pair of small scissors. Gently

pull the alimentary canal to one side so as to expose the ventral diaphragm, which will be observed pulsating strongly backward. Next cut a small hole in the top of the thorax and insert into it a drop of some stain, such as carmalum in a water solution. Almost immediately the color will appear in the ventral sinus of the abdomen, where it is forced backward by the wave-like vibrations of the ventral diaphragm. From the sinus it goes upward from the lateral openings through well-defined spaces between the air sacs and the alimentary canal and particularly up wide channels against the lateral walls of each segment. It will be noted in this experiment that the blood enters the abdomen from the thorax by way of the ventral sinus only, and that it has very definite courses through the abdominal cavity.

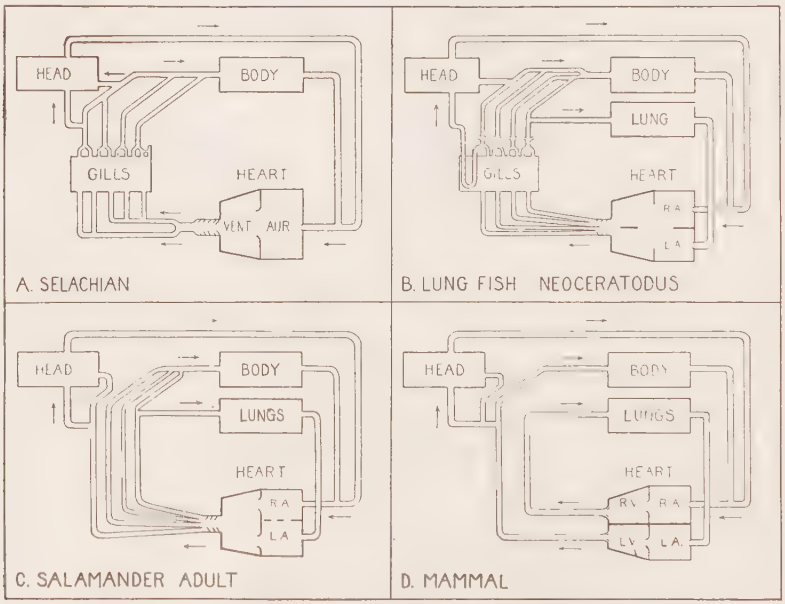


FIG. 24.—Diagram illustrating the general course of circulation in: *A*, Selachian; *B*, lung fish; *C*, adult Salamander; *D*, mammal.

The dorsal circulation cannot be observed in this same specimen because the back is removed. Therefore, take another bee and fasten it in the same manner, but now make simply a shallow median slit through the body wall of the back so as to expose the dorsal sinus and the heart from above. Insert a drop of stain into the thorax as before. After about 2 minutes this will appear in perceptible amount in the dorsal sinus, very much diluted with the blood, but in sufficient amount to give white blotting paper a distinct tint. In a short time, however, the heart becomes filled with the stained blood and appears as a colored tubular band along the median line. The blood, therefore, driven up the lateral and visceral channels of the abdomen from the ventral sinus by means of the rearward contractions of the ventral diaphragm, is drawn into the dorsal sinus through the openings along its sides by the contractions of the dorsal diaphragm, which expand the sinus. Within the sinus it is driven ahead, along the

sides of the heart, by the forward pulsations of the diaphragm, and soon it enters the heart itself by way of the lateral ostia to be pumped anteriorly through the aorta and finally out into the cavity of the head. From the head it makes its way backward again through the thorax and once more enters the ventral sinus of the abdomen. Thus the blood has a definite and rapid circulation through the larger spaces of the body cavity. But it also bathes all the tissues of the body and penetrates all the appendages, though it perhaps does not move actively in all parts or flow through the appendages. In many places the interchange of nutrient substances and waste products in solution is probably accomplished largely by diffusion.

In Fig. 24 is illustrated that series of changes which marks the evolution from the aquatic vertebrate, breathing by gills, through the conditions represented by the Lung fish and the adult Salamander to that found in the land mammals. Among the changes occurring should be noted:

1. The more efficient blood supply for the head region. This can certainly be correlated with the gradually increasing importance of the head end of the animal, and its highly specialized metabolic processes.

2. The substitution in part or in whole of the lung in place of the gills as respiratory organs. This change of respiratory habit naturally involves changes in the branchial arch blood vessels, and leads by gradual steps to the definite division of the heart into right and left halves, the right side propelling the blood through the respiratory apparatus, and the left sending the blood through the general somatic circulation.

Components of Vascular System of Individual.—Conditions similar to those which have been suggested as possible in the evolution of the vascular systems of the higher animals occur in the life history of every individual. These occur also in living forms along the line from the lower metazoa up to the higher conditions of development, which in these various adult forms are comparable to some of the developmental stages which occur in the life history of an individual.

In the higher animals the vascular system consists of:

1. *Capillaries.*—These are endothelial tubes and are usually provided with a nerve supply. These tubes branch and anastomose, and form in the higher animals a very complete network of minute tubes. In lower forms this network is less complete and is less effective. There appears to be no difference in structure between the capillaries which connect with arteries and those which connect with veins.

2. *Arteries.*—These are continuations of the endothelial tubes to which thick layers of smooth muscle cells have been added, together with some connective tissue. There is a progressive increase in thickness of the muscle and the connective-tissue layers and in the diameter of the lumen of the artery as it passes from the capillary toward the heart. The prime function of these vessels is to convey blood from the heart to the capillary system.

3. *Veins.*—These are tubes which are also continuations of the endothelial capillary tubes. They contain in their walls fibrous or elastic tissue coats which have been added to the endothelium. On the inside of the tube there are specializations of the tissues forming the wall to make valves. There is in the wall of the vein a somewhat more limited amount of muscle than in the arterial vessels. The function of these tubes is to carry blood from the systemic capillary system.

In order to keep the fluid medium in active movement there is:

4. *A Heart.*—This is a modification of a main arterial vessel of such a character that by the contractions of its walls the blood is kept in motion in a (usually) constant direction. Valves are usually present to direct the flow of the blood. In all of the higher animals and in many of the lower ones the heart becomes a complex organ upon which is imposed the duty of forcing the body fluid not only through the mass of body tissue or soma, but through the purifying apparatus as well.

Historical Statement.—The existence of heart, arteries, and veins in the bodies of the higher animals has been known for thousands of years, though the significance of their presence was long unsuspected. There probably has been no greater single advance in the history of physiology than the discovery by William Harvey of the circulation of the blood. This discovery—almost purely a study in anatomy, as contrasted with the physical and chemical investigations of the present day—paved the way for an understanding of the processes of nutrition, respiration, secretion, and excretion. Harvey had presented some of his views in regard to the circulation of the blood to medical students as early as 1615 but did not publish his full account until 1628. This was in the form of a communication to the Royal Society of London. In this work he demonstrated:

1. That the heart passively dilates and actively contracts.
2. That the auricles contract before the ventricles.
3. That the contraction of the auricles forces the blood into the ventricles.
4. That the arteries dilate passively.
5. That the heart is an organ for the propulsion of the blood.
6. That in passing from the right ventricle to the left auricle the blood transudes the lung.
7. That the quantity and rate of the passage of blood through the heart make necessary a return of blood to the heart.
8. That the blood returns to the heart by way of the veins.

Harvey was not sufficiently fortunate actually to see the blood passing from the arteries to the veins. This was reserved for Malpighi (1660) and for Leeuwenhoek (1668). From the time of his report to the Royal Society to the present, Harvey's findings have not been successfully controverted. Certain additions have been made which render our knowl-

edge of the facts of circulation more complete. The result of all these studies is that it is now known that in almost all animals above the Cœlenterates and lower worms there is some sort of circulation of body fluid along more or less definite channels, of such a sort as to carry oxygen and food materials to all regions of the animal, and to remove from the regions of the various tissues the accumulating wastes.

Incomplete Systems.—The vascular systems considered in the discussion thus far are such as consist of systems of closed tubes, heart, arteries, arterial capillaries, capillaries, venous capillaires, and veins. In the vascular systems of some animals, a considerable part of the tube system mentioned (particularly the capillaries and veins) is lacking, and is replaced by blood sinuses. These sinuses are broad cavities without definite boundaries, in which the blood washes freely the various organs and tissues after it has left the open ends of the branches of the arterial tree, and gradually makes its way back toward the heart, being continually forced on from the rear by new supplies of blood emerging from the arterioles. As the blood passes back from the sinus to the heart it passes through some organ of respiration, in which carbon dioxide is removed and oxygen added to the blood.

Functions of Different Parts of Vascular System.—The main object of the vascular system is to bring food and oxygen to the body cells and to convey away their wastes. The vessels immediately responsible for this work are the capillaries. In order to serve the desired ends, the capillaries must penetrate to all most distant parts of the organism, and come into close association with individual tissue cells. This they do. The walls of the capillaries are also thin and permeable so as to serve as diffusion membranes. While thin and permeable, they are also sufficiently strong to bear the tension of the blood stream which is being forced under pressure through the vessels.

Arteries.—The arterial vessels serve as channels along which the blood travels from the heart to the capillaries. They have a dynamic part to play in the matter of the blood flow. Through the elasticity of the fibers surrounding the endothelial tube the arteries force the blood on through channels of constantly decreasing diameter. As the blood is forced into the arteries by the contraction of the heart, the muscle fibers of the arterial walls are stretched. The pressure exerted upon the blood by the tension of the muscle fibers is known as blood pressure. This pressure is due to two chief factors:

1. The peripheral resistance in the smallest arteries and capillary system.

2. The contraction of the muscle fibers in the arterial wall.

Primary Function of the Heart.—The chief work which the heart has to do in the animal body is to force blood into the arteries against the back pressure caused by the contraction of the muscle fibers of the arteries and

arterioles in their attempt to force blood onward through the finest tubes of the blood system. This statement appears to apply equally well to contractile tubes such as occur in the worms which cannot truly be termed hearts, and to the most highly organized contractile organ found in the mammal. The back pressure of the blood in the arteries may be very low indeed, as it is among all the lower orders of animals, or it may be very high in such animals as have a highly developed arteriole and capillary network through which the blood must be forced at considerable speed.

The rhythmic contractions of the dorsal blood vessel of the earthworm are certainly analogous to the beating of a heart, even though the structure may not be homologous to a heart. In the earthworm the contraction of the vessel appears to begin at the posterior end of the body and to travel forward as a wave of contraction which practically obliterates the lumen of the blood vessel for a space of six or eight body segments. This wave of contraction drives before it the hæmoglobin-containing fluid. In the anterior portions of many of the worms there are enlarged lateral vessels branching off from the dorsal blood vessel in each of several segments. These enlarged vessels, aortic arches, or hearts are also rhythmically contractile and assist in propelling the blood through the subintestinal vessel.

Among the Mollusca, true hearts are found. In many of the lower and less active forms the hearts are very slow in action. For example, Koch found that the heart of the clam Anodonta is exceedingly sluggish. At 0°C. a single heart period may occupy as much as 2 minutes and 17 seconds. At 15°C. the heart beats only two to four times per minute. Movements by the animal do not interfere materially with the rate of heart action, but stimuli from without may cause the heart action to become very irregular. There is, therefore, a very intimate relation between vascular and nervous systems. The definite character of this relationship will be considered a little later.

It is assumed that the student is acquainted with the general structure of the heart of the frog. The heart consists of a sinus, the right and left auricles, a ventricle, and the bulbus arteriosus. Blood from all parts of the body is returned to the sinus venosus, whence it passes into the right auricle. By the pulmonary veins, blood passes from the lungs into the left auricle. The two auricles contract approximately simultaneously and expel the blood into the ventricle. The two blood streams do not readily mix in the ventricle because of the muscular meshwork within its cavity. When the ventricle contracts, the venous blood on the right side of the ventricle is the first to enter the bulbus arteriosus. Within the bulbus is a spiral valve which directs the blood into the pulmocutaneous arch leading to the pulmonary artery or arteries and cutaneous arteries. When these arteries become well filled with blood, the spiral valve is pushed over by the force of the ventricular contraction and closes the

orifice of the pulmocutaneous arch. The blood is now forced into the systemic and the carotid arches. Most of the blood passes into the systemic arch because the resistance is less in that direction. As the systemic arch becomes filled and the resistance to flow is increased in that direction, the last blood to flow from the ventricle is forced out along the carotid arches. This blood comes from the left side of the ventricle and is the purest blood in the system. The direction of flow of the blood is maintained by two sets of valves. The first are the auriculo-ventricular valves hanging between the auricles and the ventricle. The second set of valves are the pocket-shaped valves. In Fig. 25 there is shown the

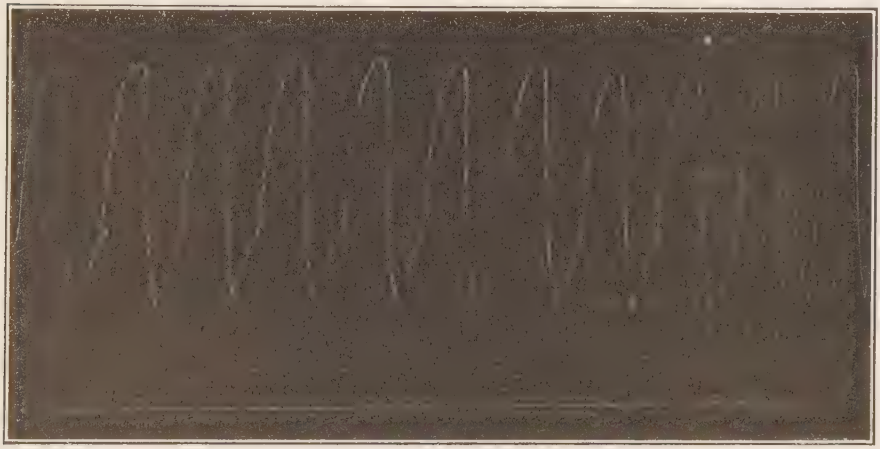


FIG. 25.—Kymograph record of normal heart beat of frog. In order to make this record, the body wall of the ventral surface was opened, the sternum removed, and the heart drawn out of the pericardial chamber. A small hook was placed in the apex of the ventricle and connected with a heart lever. The lever falls to its lowest point when the heart is completely relaxed. The very short, approximately level portion of the curve occurs during the pause between contractions. The first sharp up stroke records the simultaneous contractions of the sinus and auricle, followed immediately by the contraction of the ventricle. The record reads from left to right. $\times \frac{2}{3}$.

record of the heart of a frog beating as it lies exposed in the body of the animal.

Functions of Capillaries.—The chief function of the capillary is to serve as the medium for the exchange of substances between the blood and the lymph which immediately surrounds the tissue cells. The substances subject to transfer through the membrane constituting the capillary wall are very varied and include water, oxygen, carbon dioxide, inorganic salts, organic salts, and even colloidal substances. Water diffuses readily in either direction.

As far as the gases are concerned, it seems probable that the process involved is ordinary diffusion and the force is diffusion pressure. The diffusion in each case proceeds from a region of higher to a region of lower concentration.

Proteins do not normally diffuse out from the capillaries in most tissues. In certain tissues, however, it was found by Starling that the capillaries are normally permeable to proteins to such an extent that the effective osmotic pressure becomes lower than the blood pressure in the capillary and there is a filtration of lymph through the capillary wall as a result. This is particularly true in the case of the capillaries of the liver and to a certain extent in the case of the capillaries of the mucous membranes of the intestines.

The value of the system of capillaries to an organism will depend upon:

1. The richness of their distribution to the various tissues of the animal body.

2. The amount of surface exposed for diffusion.

3. The rate at which fresh blood can be brought into contact with the diffusing membrane.

The data presented here concerning the physiology of the capillaries are taken from Krogh's book, "The Anatomy and Physiology of Capillaries," published by the Yale University Press.

While the distribution of capillaries in the tissues of the cold-blooded animals is by no means as complete as in the tissues of the warm-blooded animals, the number seems at first thought to be very large. In a cross-section of frog muscle it has been shown that there are about 400 capillaries per square millimeter. In a similar area of horse muscle there were about 1400 capillaries, in dog muscle about 2600, and in guineapig muscle more than 4000. Krogh calculates that 1 cubic centimeter of blood in passing through the capillaries of a frog muscle has a surface contact of 2700 square millimeters; of horse, 7300; of dog, 5600. This means that diffusion can take place with great rapidity. The capillary system of organs other than muscle is even more highly developed with correspondingly increased surfaces for diffusion.

Krogh has also made it clear that the capillaries are not all distended with blood at all times. It is perfectly evident that muscles, glands, etc., vary in the amount of work which they do at different times. They do not at all times, therefore, need the full supply of blood which would be demanded when they are at their maximum activity. Krogh presents evidence showing that contractions and relaxations of capillaries may take place independently of the contractions and relaxations of arteries and arterioles. These contractions and relaxations occur as the results of the movements of certain cells (Rouget cells) which lie upon the capillary wall (see Fig. 26). The nuclei of these cells are quite large and prominent. On a dilated capillary the protoplasm surrounds the nucleus as a continuous layer on the capillary wall but diminishes in thickness toward the periphery. The margin of the cell is very irregular and sends out numerous fine branches which extend around the capillary wall. On capillaries which are partly contracted the protoplasmic mass surrounding the nuc-

leus is somewhat thicker, and the contours of the cell processes are more distinct. In extreme contractions the protoplasm is tightly packed about the nucleus and the branches are short and stout. In some cases the cell processes anastomose so as to form a sort of network surrounding the capillary.

Dr. Vimtrup has shown that the contraction of the capillaries is due to the movements of the Rouget cells, and Rehburg has shown that the endothelial lining of the capillary is folded during strong contraction.

An attempt to combine all the observations into a coherent conception of the normal anatomy of a capillary will give about the following result: Like the walls of the smallest arteries and veins, the capillary wall consists of two distinct elements—the endothelial tube and the outside muscular coat. The important difference between capillaries and larger vessels lies in the arrangement of the

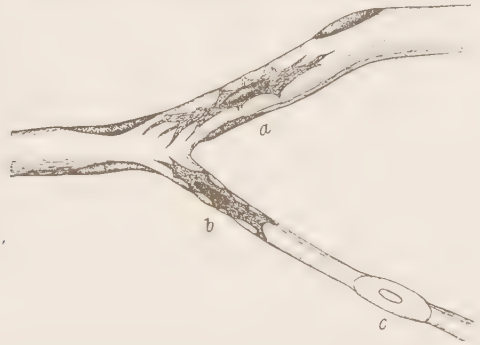


FIG. 26.—Two Rouget cells (*a* and *b*) as seen on capillaries in living newt larvæ. *b* is contracting, *c* is a red corpuscle. (*From Krogh's "Anatomy and Physiology of the Capillaries." By permission of the Yale University Press.*)

muscles, which in the arteries and veins form a more or less continuous layer, greatly increasing the thickness of the wall and offering a considerable resistance against the exchange of substances between the blood and the surrounding lymph spaces or tissue cells, while in the capillaries the muscular coat is arranged more or less in the form of a wide-meshed network, leaving the larger part of the endothelial surface uncovered and adapted for the passage of substances with a minimum of resistance.

The muscular coat of the capillaries possesses, like smooth muscles generally, a definite tonus, or *posture,* in the terminology of Sherrington (1920), subject in the organism to nervous, hormonal and other influences, and the diameter of a capillary is, on the whole, determined by the state of contraction of its muscular coat. The changes observed in the configuration of the endothelial cells and in the shape of their nuclei make it clear, however, that the endothelial tube itself must possess some normal form and caliber which it will assume when the outside and inside pressure is absolutely the same. When the outside pressure is the higher, the tube collapses. When the Rouget cells contract, it becomes folded, and when they relax and the inside pressure is ever so slightly higher than the outside, it is dilated, and the endothelial cells are passively stretched,

their surface is enlarged, their thickness is diminished and their nuclei are flattened out. We have here a close analogy to the behavior of the alveolar epithelium in the lungs of man, according to Marie Krogh's (1915) determinations of the pulmonary diffusion constant. These determinations of the quantity of carbon monoxide which will diffuse into the blood from the alveoli show that when the lungs are expanded beyond a certain point the epithelial surface becomes larger and thinner by stretching, but when they are allowed to collapse below that point the surface area and thickness remain constant—the surface is folded.

The capillaries are also provided with definite nerve control. It has been shown by Hooker that stimulation of the cervical sympathetic of the cat induces a pronounced contraction of the capillaries of the ear. Krogh has made the same observation in the case of the rabbit. In the frog, Krogh and others have shown that stimulation of the lower ganglia of the sympathetic chain brings about constriction first of arteries and then of capillaries of the web. Krogh is of the opinion that every single Rouget cell is supplied with a sympathetic fiber and can be made to contract through it. Removal of the sympathetic ganglia in the frog, or cutting the sciatic nerve below them, results in a more or less permanent dilation of the capillaries.

In a recent paper Parker has called attention to the work of Pomeroy, done some years ago, which showed that the worm Nereis possesses upon the walls of its blood vessels large numbers of cells, capable of being stained by methyl blue, which bear a very strong resemblance to the cells described by Krogh and figured by Vimtrup. These cells had been seen in Nereis and described by Retzius. They were considered by Retzius muscular in nature. The similarity in form which they bear to the Rouget cells leads Parker to suggest that they may be true Rouget cells, although it has not yet been demonstrated that in Nereis they are contractile. If it can be shown that they are contractile, and that they do actually control the diameter of the blood vessels, it would appear that a definite comparison may be drawn between the blood tubes of the higher worms and the capillaries of the vertebrates.

Functions of Sinuses and Veins.—These channels have apparently the purpose of receiving the blood forced out from the ends of the arterial vessels and of returning it to the heart again, thus completing the circuit. Among the invertebrates the sinuses are merely spaces among the muscles and viscera through which the blood passes upon its way from the ends of the arteries to the aerating organ. They serve as lymph channels and bring the circulating fluid into close communication with the body tissues. Among the higher animals there are examples of sinuses in the tissues covering the brain. Here the duramater forms the outer wall of the sinus.

The Elastic Tube System.—The tubes forming the vascular systems of all animals have elastic walls. This fact is of great importance in the

life of the animal. In any system of tubes with rigid walls as much fluid must escape in any unit of time from the distal end of the tube system as is forced into it at the proximal end. The blood, like water, has only a very minute degree of compressibility, and at the low pressures which exist in the vascular systems of animals this amounts practically to zero. When the heart (ventricle, in higher forms) contracts, the energy of its contraction is expended in stretching the muscular fibers of the arterial walls as more fluid is forced into a tube which is already filled with blood. The extensibility of the muscle fibers and their elasticity obviate to some degree the most extreme variations in pressure which would occur in a system of rigid tubes. Also the blood is being constantly forced onward through the arteries and arterioles because of the tonic contraction of these muscle fibers. The capillary walls are, therefore, relieved of those sudden variations of pressure which might readily lead to their rupture, loss of blood in tissue spaces, and finally failure of the vascular mechanism.

Peripheral Resistance.—The resistance met by the blood as it flows through the arterial vessels is the friction of the fluid against the walls of the narrow tubes. The smaller the diameter of the tube the greater the friction between the wall of the tube and the liquid being forced through it. It will be seen readily that this resistance in the vascular tubes will be greater the more finely divided the arterioles become. In animals in which the arteries never become finely divided and which finally end by opening into a blood sinus, the resistance to the flow of the blood is decidedly less than in forms in which there is a complete closed system of tubes, and in which the arterioles are of very small diameter. In order that the blood may be propelled through the tube system, it is essential that the pressure of the blood against the arterial walls be high enough to overcome the peripheral resistance. This means that in the higher animals with completely closed systems of tubes, and with fine arterioles and capillaries, the pressure must be much greater than in animals which have only a partial tube system and low resistance.

Peripheral resistance in a given tubular system may be varied by the degree of contraction of the muscle fibers in the walls of the vessels. The degree of tension may be varied by nervous stimulation through the vasomotor nerves or by the presence or absence of certain chemical substances, such as adrenalin in the blood—or by variations in the ratio of certain salt components (*e.g.*, Na, Ca) of the blood plasma.

CHAPTER XIV

PHYSIOLOGY OF THE HEART

Investigators who have worked upon the effects of metallic ions upon the heart and its activities have been confronted with the necessity of maintaining a continuous supply of oxygen for the use of the hearts under experimentation. This oxygen has been provided in a variety of ways: by immersing the heart in a saline solution through which oxygen gas is slowly bubbling; by perfusing the heart with a saline solution in which oxygen is dissolved; by immersing in or perfusing with a saline solution containing a small amount of hydrogen peroxide. In case the hydrogen peroxide is used, care must be taken to restore to the proper balance the hydrogen ions of the solution, since hydrogen peroxide as sold usually contains a considerable amount of acid. The addition of small amounts of sodium bicarbonate solution to the saline will restore the solution to its proper hydrogen-ion concentration.

Relation of Oxygen to Heart Beat.—The need for oxygen is probably twofold: First, oxygen is doubtless needed for the building up within the muscle cells of the substance known as the lactic acid precursor (hexose phosphate), which appears to be essential for contraction; second, oxygen is needed for the respiratory—and possibly other processes of the nerve cells upon whose activity the initiation and maintenance of the beat depends.

In Fig. 27 is shown the record of the isolated heart of a crab beating in a solution very similar to sea water. This record reads from right to left. At the start the heart was beating in a solution containing oxygen. At *b*, it was placed in a solution lacking in oxygen. The beats became almost immediately of varying amplitude and decreased in amplitude very rapidly. At *c*, the heart was again immersed in a solution containing oxygen. For a few minutes there was no perceptible increase in the amplitude of contraction, but later the contractions resumed practically their original amplitude, force, and regularity.

Similarly, it can be shown that the beating of the isolated heart of a frog can be modified by the presence or absence of oxygen in the bathing solution. In Fig. 28 is shown a record of the effect of the presence and absence of oxygen upon such a preparation. The record reads from left to right. At *a*, the heart was beating in Mines' solution containing oxygen. At the point indicated by the arrow the heart was placed in a sample of the same solution lacking in oxygen. During this time the

FIG. 27.—Effects of oxygen upon heart beat of a crab. Portion of the record made by a heart of a crab beating in a solution containing 100 cubic centimeters $\frac{3}{8}$ *M* NaCl, 2.2 cubic centimeters $\frac{3}{8}$ *M* KCl, 7.8 cubic centimeters $\frac{3}{8}$ *M* $MgCl_2$, 3.8 cubic centimeters $\frac{3}{8}$ *M* $MgSO_4$, 3.25 cubic centimeters $\frac{3}{8}$ *M* $CaCl_2$, 0.75 cubic centimeter $\frac{3}{8}$ μ $NaHCO_3$. The beats shown at *a* are characteristic for hearts beating in such a solution and under ordinary conditions will continue for 30 hours or more. At the point *b* the solution containing oxygen was replaced by one which had been heated to expel the gases in solution and then cooled to the same temperature as the solution first employed. The effect of the lack of oxygen is shown in the regularly decreasing force of contraction. At *c* the heart was again placed in the solution containing oxygen, and after a short time regained its former strength and continued beating for a number of hours. This experiment was repeated frequently with similar results.

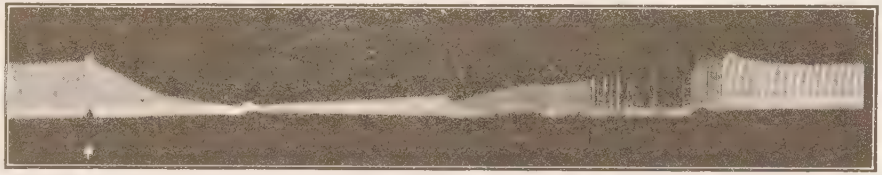


FIG. 28.—Effect of oxygen upon isolated heart of frog. *a* Heart beating in Mines' solution of *M* 8 concentration and oxygen. *b*. At arrow, heart was placed in *M* /8 Mines' solution lacking in oxygen. *c*. Heart placed again in Mines' solution and oxygen.

amplitude of the beats, particularly of the ventricle, decreased very rapidly. At *c*, the heart was again placed in the solution containing oxygen. The recovery was somewhat slow and irregular, but after a few minutes the amplitude, rate, and force of contractions were fully restored.

Garrey's work upon the heart of Limulus shows very beautifully that, in that form at least, the process involved in the initiation of a heart beat results in the production by the ganglion cells of carbon dioxide, and is, therefore, very probably an oxidative process. As such it is doubtless carried on by some form of enzyme. The temperature coefficient of the reaction is such as would lead one to suppose it to be a purely chemical reaction.

Effect of Changes of Temperature upon Heart Action.—The suggestion has been made that something may be learned as to the nature of a physiological process from the study of the effect of changes of temperature upon that process. It is now a matter of common knowledge that for a rise of temperature of 10°C. ordinary chemical reactions are increased in rate from two to three or more times. If any physiological process shows essentially the same modification in rate by changes of temperature as do ordinary chemical reactions, the belief would seem to be justifiable that some chemical reaction lies at the basis of the physiological process.

In the higher animals heart action takes place in an environment which has an almost constant temperature. Among the reptiles, amphibians, fishes, and all invertebrates the body temperature fluctuates with the temperature of the surroundings. The body processes of the higher animals have become so adjusted to the constant temperatures at which they normally take place that any marked change of temperature may result in a serious disturbance of the whole organism. On the other hand, the poikilothermous animals are constantly subjected to variations of temperature in their daily life, and unless these variations are extreme in amount they cannot be held to be unusual for the animals concerned. Observations of the heart rates of cold-blooded animals within the range of temperatures to which they are normally subjected ought therefore to give valuable information as to the general nature of the reaction or process underlying cardiac activity.

Experiments to determine the effect of changes of temperature upon the heart may be performed in a variety of ways: The whole animal may be subjected to temperature changes by changing the temperature of the environment in which it is placed, or the heart may be removed from the body of the animal and be allowed to beat while suspended in a saline solution, the temperature of which can be definitely controlled. If the whole animal is subjected to temperature modification, it is important to know the temperature relations existing between the animal and its environment. In small forms such as fish embryos, small Crustaceans,

etc., there can be little doubt that the temperature of the water fairly represents the temperature of the animal body. In such forms as the earthworm and the small spotted salamander *Diemyctylus* it has been shown that within a few minutes the body temperature comes to be the same as the environing temperature to within a very few thousandths of a degree Celsius. In such forms as large, freshwater clams and goldfish there is a larger bulk of material to become adjusted and the process takes longer—perhaps half an hour to an hour— but in the end there is the same close approximation of the body temperature to that of the surroundings.

The *temperature coefficient*, expressed by the symbol Q_{10}, may be calculated by the use of the formula,

$$Q_{10} = \left(\frac{K_1}{K_0}\right)^{\frac{10}{T_1 - T_0}},$$

in which K_1 = rate at the higher temperature T_1,

K_0 = rate at the lower temperature T_0.

Table 30 gives an indication of the values of Q_{10} as determined by various investigators upon the hearts named.

TABLE 30.—TEMPERATURE COEFFICIENTS OF HEART ACTION

Animal	Range in degrees Centigrade	Q_{10}
Tubifex	9–16	3.213
Earthworm—average 35 experiments	9.5–27.5	2.173
Nereis—average 14 experiments	3.2–18.5	2.525
Phyllorhoea	16–30	2.5
Maja verrucosa	7–26	3.0
Fundulus embryo—average 22 experiments	6.5–21.5	3.081
Fundulus embryo—average 14 experiments	22.52–33.78	1.521
Pacific terrapin ventricle	2.0–32	2.5
Rana esculentula ventricle	13–24	2.8
Man fever	37–42	2.2

The figures presented in the table would appear to indicate that the controlling fact in the rate of heart beat in all of the animals mentioned is some chemical process. The question may fairly be raised as to what such a reaction may be and where it may take place. Garrey's work upon the heart of Limulus is certainly conclusive for that form and strongly indicative of what may be expected in other forms—that the origin of the heart beat is the sequel to a chemical process which results in the formation of carbon dioxide in the ganglion cells of the heart. In Fig. 29 is given the record made by a beating isolated frog heart under the influence of Ringer's solution at temperatures of 9 and 24°.

The Origin of the Heart Beat. — It is a matter of common laboratory experience that the hearts of many (not all) animals, especially of the cold-blooded types, may continue to beat for shorter or longer periods after they have been removed from the bodies of which they were parts. This may readily be demonstrated by simply suspending the heart of a frog or turtle in a tube of Ringer's solution, or, even better, by so suspending the heart attached to a perfusion apparatus that there is a flow of the solution through the heart chambers.

It is, of course, evident that the continuance of the beat is not due to any connection of the heart with the central nervous system. The hearts of such animals as the crayfish and some of the crabs do not so readily

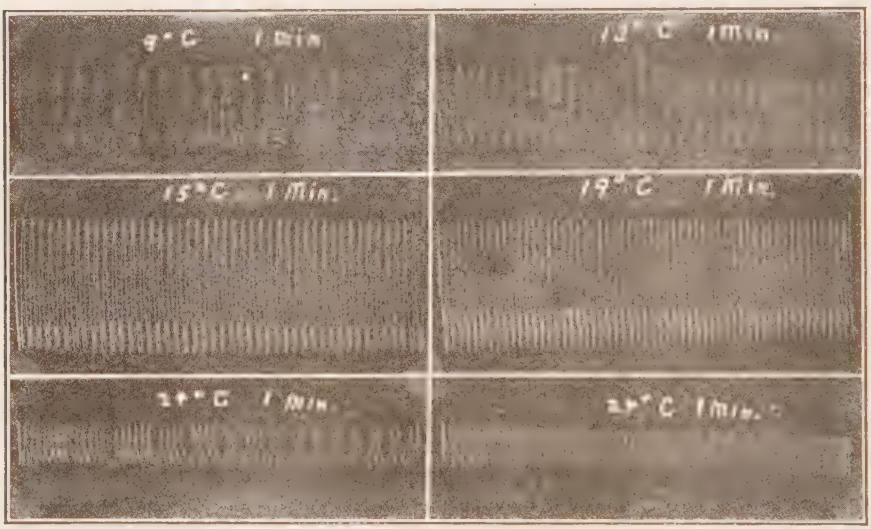


FIG. 29.—Effect of temperature upon the action of heart. The temperature coefficient in this case is 2.184. (*Record made by Courtland Griswold.*)

survive such removal. (This may be because of the severing of some important nervous connections.)

Physiologists have raised question as to whether, first, the rhythmic activity of the heart muscle is due to some rhythmic power located within the heart muscle fiber itself or, second, whether it represents simple responses on the part of the heart muscle to stimuli arising in some portion of the nervous system and transmitted to the heart muscle through the nerve fibers. It is a well-established fact that there are found in all animals, certainly from the Mollusca and Crustacea upwards, nerve cells and fibers located in various parts of the heart wall; and that these have something to do with the regulation of the beat. Such regulation of the beat will be discussed in another section.

As far back as the time of Haller it was recognized that the origin of the beat of the heart is in some way dependent upon the composition of

the blood—or of the fluid bathing the tissues. In the years since Haller a large number of investigators have attempted to solve the problem of the origin of the rhythmic contraction of the heart. Among these may be mentioned Merunowicz, Ringer, Locke, Howell, Lingle, Loeb, Mines, Clark, and others. Among the most recent workers in this line are Andrus and Carter. The answers which have been given in the solution of this problem include a great variety of possibilities, such as:

Myogenic Theory of Heart Beat.—The evidence in favor of the idea that the rhythmic activity of the heart may be inherent in the muscle fibers is of several sorts.

1. Tissue cultures of heart muscle cells removed from chick embryos and kept in blood plasma show regular contractions over extended periods of time, even though they are not in connection with any nervous structures.

2. The heart is one of the first structures to be elaborated in the embryonic development of any of the higher animals, arising soon after the elements of the nervous system are laid down. In chick embryos and in fish embryos the heart begins its rhythmic movement, presumably, before any connection of nervous elements of the heart musculature have become established. Of course, it may be true that neuroblasts migrate into the heart wall at a very early period, take up positions, and establish connections before heart action begins, but this has not, as far as the author is aware, been established. The fact that an embryonic tissue might take on a habit of contracting rhythmically does not of necessity mean that the adult tissue would continue the habit.

3. The importance of the inorganic constituents of the perfusing fluid in bringing about rhythmic heart action has been emphasized. Included here would be the work of Ringer, Locke, and the large group of those who have sought in the balance of the various salts in the blood stream the explanation of the rhythmic activity of the heart.

Rogers showed some 20 years ago that the heart of the crab Brachynotus would continue to beat for a considerable period in an artificial solution of the proper composition. The heart of this animal does not at once cease beating when it is removed from the body. Hence, it is impossible to say whether any particular substance is essential for the origination of the rhythmic contractions. The survival of the rhythmic contractions of the heart is, however, dependent upon the composition of the solution. It was demonstrated that rhythmic contractions will not continue long when sodium chloride is absent. It was also known that calcium, potassium, and magnesium each have an important influence upon the heart contraction. Sodium chloride has the power to restore rhythmic contractions in hearts which have ceased beating in other solutions. Hearts which have ceased beating in a pure sodium

chloride solution do not again beat when placed in a solution lacking in sodium chloride. It is apparent also that the balance existing between the salts entering into the composition of the artificial solution, and pre-

All records read from right to left. The time marker indicates intervals of 30 seconds.

FIG. 30*A*.—Record of a heart of a crab beating in a solution containing 100 cubic centimeters $\frac{3}{4}$ *M* NcCl, 2.2 cubic centimeters $\frac{3}{4}$ *M* KCl, 7.8 cubic centimeters $\frac{3}{4}$ *M* $MgCl_2$, 3.8 cubic centimeters $\frac{3}{8}$ *M* $MgSO_4$, 1 cubic centimeter $\frac{3}{8}$ *M* $CaCl_2$, 0.75 cubic centimeter $\frac{5}{8}$ *M* $NaHCO_3$ and oxygen. It will be noticed that the first beats were strong, but became rapidly weaker, then slower, finally ceasing in less than an hour from the beginning of the experiment. The curve is characteristic of all hearts beating in solutions in which the amount of Na is too great, or the Ca too small.

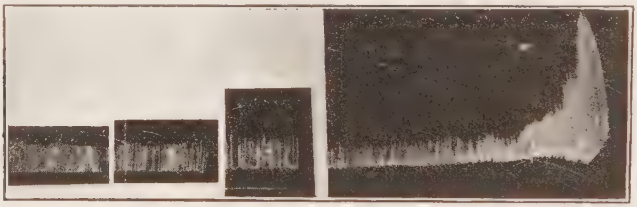


FIG. 30*B*.—Record of a heart of a crab beating in a solution similar to that mentioned above, except that 2 cubic centimeters $\frac{3}{4}$*M* $CaCl_2$ were employed. This heart beat for a much longer period than that in the previous experiment. Section *b* of the record was taken 2 hours from the start *a*; section *c* 4 hours, and section *d* 6 hours.

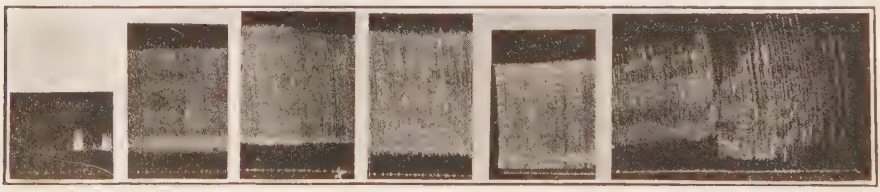


FIG. 30*C*.—Record of a heart of a crab beating in a solution similar to those used in the above experiments, except that it contains a larger amount of $CaCl_2$, *viz.*, 3 cubic centimeters $\frac{3}{4}$ *M* solution. The beats maintain their first strength and rate of contraction for a long period. Section *b* is taken 4 hours from the beginning of the record, section *c* 7 hours, section *d* 11 hours, section *e* 18 hours, and section *f* 20 hours. At the point *x* in section *d* a couple of drops of hydrogen peroxide were added to the solution in which the heart was immersed. Its effect is shown in the ensuing stronger and more rapid contractions.

sumably of the blood also, is a very delicate one and can be determined with very great accuracy. In Fig. 30, *A*, *B*, and *C* are shown fragments of records of the beating of the heart of Brachynotus in the artificial solutions mentioned.

It has been found possible to maintain rhythmic contraction in strips of ventricular muscle of the turtle for a long time by simply suspending them in appropriate salt solutions. Such strips have been carefully examined histologically and appear free from ganglion cells. Of course, nerve fibers are present. The work of Lingle shows that sodium chloride is absolutely essential for the origination of rhythmic contractions in strips of turtle's ventricle, and that oxygen is equally necessary for maintaining long-continued rhythmic action. This may be supplied as oxygen gas, or as H_2O_2. Whether Lingle's preparations of ventricular strips were entirely free from nerve elements as he believed them to be seems doubtful to the present author. Similarly, others have found that sodium chloride has a definite rôle to play in maintaining rhythmic heart activity in a great variety of hearts. In all these cases, however, it is impossible to be free from the suspicion that the NaCl may affect nerve elements in the heart wall, as well as muscle.

In a rather recent review of the relation of ions to the contraction of heart muscle, Mines again calls attention to the balance of ions which must exist in a physiological saline solution, as already indicated by Loeb. Mines found the frog heart to beat continuously and normally in a solution of the composition

100 cubic centimeters $M/8$ NaCl
2.5 cubic centimeters $M/8$ KCl
2.0 cubic centimeters $M/8$ $CaCl_2$
1.5 cubic centimeters $M/8$ $NaHCO_3$

provided the solutions are kept saturated with oxygen.

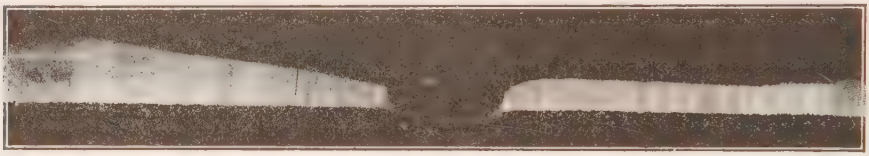
Removal of sodium from the solution renders heart contraction impossible. Sodium probably acts in a variety of ways in the solution: It serves to maintain the proper osmotic pressure, and serves also as the chemical stimulus for the muscular contraction.

Removal of potassium from the solution results in a prolonged systole. The removal of the potassium does not, however, render heart action impossible, provided the sodium and the calcium are maintained in proper balance and the needed oxygen is supplied.

Removal of calcium (see Fig. 31) results in a great weakening of the contractions, ending in what may be termed the *sodium standstill.* Under the influence of the unantagonized sodium the heart muscle contracts with greater rapidity for a time, the beats becoming of less and less amplitude until they cease entirely. At the same time the muscle as a whole loses tone and seems to stretch. This action is reversible if not allowed to go too far, since the addition of calcium to the solution or the replacing of the sodium and potassium alone by a solution containing calcium also will frequently cause the heart to resume contractions which may continue for a long period in a perfectly normal way. Just as sodium is essential for the initiation of the heart beats, so calcium or some similar

bivalent ion is necessary to overcome the poisonous effect of the sodium, and so maintain heart beats over a long period. It may be that the calcium (or other substitute for it, as strontium) forms, with some substance of the muscle (or its membrane), a compound which dissociates readily and which is a cardinal factor in the activity of the muscle. Strontium is apparently able to fill the same niche in the architecture of the muscle as the calcium. Other bivalent metal ions do not have this same power.

4. The importance of some of the products of heart-tissue metabolism has been emphasized by some. There seems to be so little in the suggestion that it is offered only as a matter of record.



a *b* *c*

FIG. 31.—Effect of salts upon isolated frog heart. *a*. Heart beating in *M*/8 Mines' solution and oxygen, pH 7.35. *b*. Heart placed in *M*/8 NaCl and oxygen, pH 7.35. Note that at the very first there is an increase in the amplitude of the contractions. This soon decreases steadily until the heart finally stops. *c*. Heart placed in Mines' solution. Beats are quickly resumed and continue for a long period. (*Record made by Courtland Griswold.*)

5. The importance of the electrical charges of certain surfaces or membranes in the heart has been mentioned by Mines. He says:

> Some essential part of the excitatory or contractile mechanism of the heart involves certain surfaces or membranes whose differential ionic permeability must be kept at or near a certain value. Since, other things being equal, the permeability of the membrane depends upon its electric charge, the electric charge must be kept at or near a certain value.

Later, Mines conceived that "the abnormalities of contraction resulting from overrapid stimulation were incident to the local accumulation of lactic acid."

6. The importance of the hydrogen-ion concentration of the perfusing liquid has attracted the notice of others. Clark first called attention to the necessity of maintaining the hydrogen-ion concentration between $10^{-6.7}N$ and $10^{-8.5}N$. Dale and Clark had shown that weakly acid Ringer's solution has the effect of reducing the force of the beat and of impairing conduction from auricle to ventricle. Mines recorded slowing of the frog's heart and the lengthening of the A. V. interval with solutions of hydrogen-ion concentration of $10^{-5.5}$, and with one of 10^{-9} a quickening of the rate and a shortening of the conduction time. Finally, Dale and Thacker demonstrated that the range of the hydrogen-ion concentration in which automatic rhythm develops in the frog's heart shifts toward the

alkaline side when passing, in testing, from the venous to the arterial end of the heart.

Careful determinations of the hydrogen-ion concentrations of the body fluids of a variety of animals appear to indicate that there is a comparatively limited range of hydrogen-ion concentrations (pH 10 to pH 5.5) which will permit heart contractions to take place. Within this range is a much narrower range which may be considered to represent the optimum concentration of hydrogen ions for the particular heart in question. Also, it has been determined in certain cases that the concentration of hydrogen ions which may be the optimum for the action of one part of a given heart may not be the optimum of some other part of the same heart. In the hearts of certain of the vertebrates the optimum for the sinus is slightly more acid than that of the auricle, and the optimum concentration for the auricle is somewhat greater than that for the ventricle.

The following chart gives an indication of the pH limits found by Dale and Thacker to exist in the heart of the frog.

TABLE 31

Concentration	pH 1	2	3	4	5	6	7	8	9	10	11	12	13
Sinus													
Auricle													
Ventricle													
Duration S. A. Interval										→ decreases			
A. V. Interval										→ decreases			
Cycle			increases ←							→ increases			

Usually the optimum hydrogen-ion concentration is slightly on the alkaline side of the neutrality, though in certain of the Ascidians it is on the acid side.

In general, it may be said that an increase in the hydrogen-ion concentration beyond the optimum tends to diminish the force of the systole, to increase the diastole, and finally to cause a rest in a state of complete relaxation. If in this completely relaxed condition the heart is now washed in a neutral solution, weak beats may occur, but if the heart is washed in a slightly alkaline solution, prompt recovery occurs. Too strong an alkaline solution will cause the heart to stop in systole. The stoppage of the heart in the more acid solution may be due to some alteration in the chemical character of the muscle substance. Whatever this change is, it may, if not allowed to continue for too long a time, be almost instantly reversed by an alkaline solution. Whether the action here is similar to that in the case of spermatozoa which are unable to move in an acid solution, but which become active in a slightly alkaline solution, is a matter for further investigation.

Still more recently E. C. Andrus, working upon the heart of the tortoise and keeping the hydrogen-ion concentration always within limits which are normal to the organism and on the alkaline side of neutrality, found that slight alterations in the pH value of the perfusate causes definite changes in the heart's activity. A decrease in acidity to a pH value of 7.6 to 7.8 brings about a rise in the tonus of the auricular muscle and the amplitude of the beat is decreased. A slight raising of the pH value to 7.3 increases the cardiac output in either or both of two ways; *i.e.*, by a decrease of tone and hence an increase in initial volume, or by an increase in the amplitude of the beat.

It is an interesting fact that the trivalent ions of the rare-earth metals La‴, Nd‴, etc., have the same effects upon the heart as an acid solution, although the solution in which they act may be perfectly neutral. The influence of the solution is remarkable, a concentration of $\frac{1}{100,000}$ normal solution of either of the earth metals mentioned stopping the heart of the frog in 30 seconds. Often concentrations of only $\frac{1}{1,000,000}$ normal stop the heart. The mode of arrest, absence of recovery, or slow and imperfect recovery on washing out with neutral saline solutions, and the immediate and complete recovery on washing out with alkaline solution are all reproduced exactly.

Andrus and Carter have experimented upon the hearts of the terrapin and upon dogs, perfusing the hearts with the desired media. Galvanometric records were made and the intervals carefully measured. As the result of their work it appears to be demonstrated that in these preparations it is possible to control accurately, to accelerate and to retard, to abolish and to restore the development of the excitatory process and its propagation by changing the pH of the perfusion fluid. In this work variations in the hydrogen-ion concentrations between pH 7.1 and 7.8 were employed. It was found that a change from a more alkaline to a less alkaline perfusing fluid is accompanied by a definite slowing of the rate, and that shifting to a perfusate of higher pH results in a more rapid rhythm. Coincident with these changes there are also observed changes in the rate of conduction from auricle to ventricle. This period is uniformly lengthened by a perfusate of pH 7.1 and shortened by one of pH 7.8. In other words, change to a less alkaline perfusate retards the rhythm of the heart as a whole and decreases the rate of conduction, while variation toward a more alkaline solution produces a more rapid rhythm and facilitates conduction.

Heart Block in Chick Embryo.—It was found by Mr. Johnstone that delicate ligatures could be placed at different levels around the beating hearts of chick embryos and that he could thus functionally isolate the different parts of the heart tube, producing what is known in the adult as *heart block*, a condition where

the subdivisions of the heart beat independently of each other, the coordination normally existing between ventricle and atrium being lost. Since this could be done in embryos of 2, 3, and 4 days' incubation, it became possible for Mr. Johnstone to study contraction of embryonic heart muscle before the problem is complicated by the ingrowth of nerve fibers, and where the only conducting system is the muscle itself. Further than that, by altering the level of the ligatures, the different parts of the heart tube could be observed, both separate and connected, and thus the effect of one part on another could be determined for different stages of growth.

Mr. Johnstone found that when a ligature is tied at the atrioventricular junction, the atrium continues to pulsate, while the ventricle and bulbus, after stopping for a moment, begin to contract, but at a slower rate and more irregularly than that portion of the heart on the other side of the ligature—that is, a definite heart block is produced. If, after the ventriculo-bulbar segment has resumed its contracting, another ligature is placed around the heart at the junction of the ventricle and the bulbus arteriosus, the latter stops contracting while the ventricle continues without apparent change. The bulbus in these experiments sometimes fails to beat again, but if pulsation is reestablished it is after a longer interval and at a slower rate than in the isolated ventricle.

At present there is some uncertainty as to the exact location of the junction of the sinus venosus with the atrium in these early stages, and this is one of the problems that now faces us. Mr. Johnstone placed a ligature in this general region and all that part of the heart tube cranial to the ligature (atrium, ventricle, and bulbus), after a brief cessation, resumed pulsations, maintaining the regular sequence of atrium, ventricle, and bulbus, though at a slower rate than before tying the ligature. It would thus appear that the ultimate and regulative control of the heart beat must be in the sinus region.

Further experiments were done in which the ligatures, after being firmly tied, were removed, leaving a crushed line of constriction in the cardiac tube that physiologically isolated the portions on either side of the constriction, producing heart block in accordance with the level at which the crushing occurred.

Dr. Lewis has studied the initiation and character of the early rhythmic contractions of the heart by removing this organ from 2- and 3-day chick embryos and observing its behavior in Locke's solution, where it maintains fairly regular automatic pulsations for several hours at room temperature (26°C.) varying in different hearts from 40 to 80 per minute, which is about one-third of that in the freshly opened egg. If the temperature is gradually increased the rate increases, going from 26 to 30°, about four beats per minute, from 30 to 35° from four to eight beats per minute, and from 35 to 40° from four to twenty beats per minute. The rate of the isolated heart varies definitely according to certain environmental conditions, such as temperature, carbon dioxide tension, hydrogen-ion concentration, and salt content of medium, and also upon mechanical stimulation.

In studying the effect of changes of temperature on contraction phenomena of these isolated hearts, Dr. Lewis found that the heart block which Mr. Johnstone had effected by ligatures could be brought about simply by an abrupt rise in temperature. For example, when a heart beating regularly at 92 per minute at 27°C. was placed in a warm box at 38°C., after 3 minutes the atrium was beat-

ing at 120 and the ventriculo-bulbar part at 12 per minute; and when returned to room temperature, within 10 minutes the whole heart was found beating synchronously again at 92 per minute. There was some variation in the behavior of the individual specimens as to the degrees of temperature necessary and the amount and character of the disharmony, but in general the effect was as striking as the illustration given. With few exceptions the atrial end was most resistant and beat most rapidly and the ventricular and bulbar portions, in that order, exhibited a much slower rhythm and showed more tendency to be intermittent. The heart block of the clinicians has been supposed to be due to injury to the atrioventricular conduction bundle. Since these young embryonic hearts contain no such bundle, it cannot be the explanation here. Nor is it due to any serious injury of the atrio-ventricular or ventriculo-bulbar junctions, since the condition disappears when the hearts are brought back to room temperature. The explanation must rest in the physiologic differences existing between the muscle cells of the atrium, the ventricle, and the bulbus. Apparently these physiologic differences are increased through the circumstances of the experiment and the different parts of the heart are rendered more sensitive to thermal and other changes. It is certainly not temperature alone that causes heart block, because the same temperature in the egg, with the heart in the normal environment, yields a synchronously beating heart.

The inherent difference in the constituent cells of the atrial, ventricular, and bulbar portions of the cardiac muscle sheet is best shown by dividing the isolated heart into these three parts and studying them in Locke's solution. Dr. Lewis made records of such preparations, lying side by side, under various conditions of temperature and in open and closed chambers. This latter point makes a difference, for when the observing chamber is closed with a cover glass the rate is accelerated, due presumably to increased carbon dioxide tension. It is hoped that this important point can be definitely determined. When kept at room temperature it was found, in confirmation of Mr. Johnstone's ligature experiments, that the atrium always beats faster than the ventricle and faster than the bulbus. The rate of the atrium approaches closely that of the entire heart. The rate of the ventricle, on the other hand, is usually less than half that of the atrium for the 2-day hearts, and considerably less than that for the 3-day hearts. The ventricular rhythms are apt to be more irregular than the atrial. The pulsations of the bulbus often fail entirely and are usually few in number and irregular when they do occur. All of these things make it quite evident that the relatively fast rate of the ventricle and bulbus in the intact heart is dependent upon stimulus from the atrium. When these parts are separated they show less spontaneity in older stages than younger ones. In other words, the muscle cells of the ventricle and bulbus tend to lose their spontaneous rhythmicity with age.

Neurogenic Theory of the Heart Beat.—The evidence for a belief in the nervous origin of heart contraction comes in part from a study of the distribution of nervous elements in the musculature of the heart. It is found that the degree to which rhythmic power is developed in the heart muscle is evidenced by a corresponding development of the nervous structures of the heart wall. The nervous elements are especially abundant

in the posterior (or sinus) part of the heart tube (in amphibian and reptile) and least so in the apex of the ventricle. Such a distribution is fairly characteristic of the conditions found among the vertebrates. It may be suggested that the different character of the muscle cells in the different regions of the heart wall should be looked to to explain the differences noted

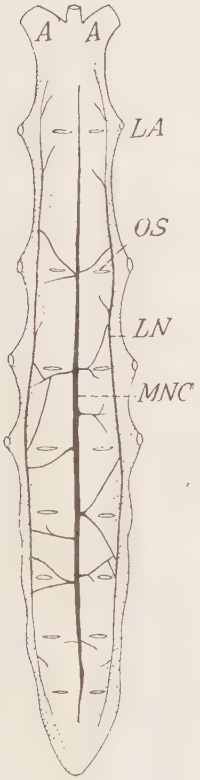


FIG. 32.

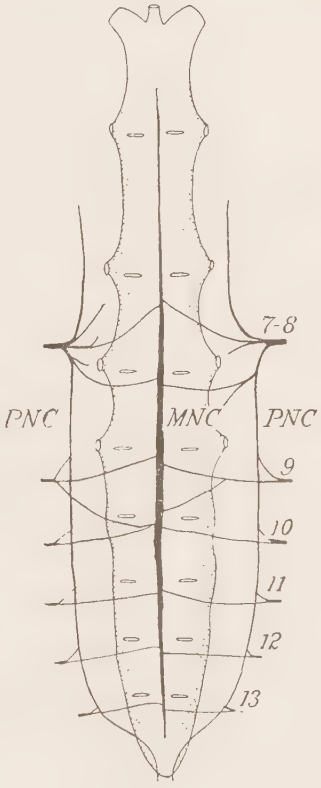


FIG. 33.

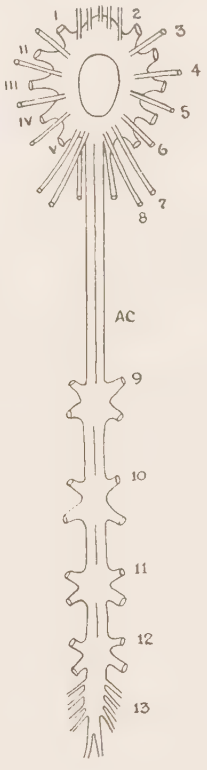


FIG. 33A.

FIG. 32.—Heart and heart nerves of *Limulus polyphemus*. Dorsal view. *A*, anterior artery; *LA*, lateral ateries; *LN*, lateral nerves; *MNC*, median nerve cord; *OS*, ostia.

FIG. 33.—Heart of *Limulus*. Dorsal view. Showing the connection of the ventral with the cardiac nervous system. *MNC*, dorsomedian nerve cord on the heart; *PNC*, pericardial nerves; 7, 8, branches from the two posterior hæmal nerves from the brain; 9–13 branches from the hæmal nerves of the abdominal ganglia.

FIG. 33*A*.—Brain and abdominal ganglia of *Limulus*. Dorsal view. I–V, nerves to corresponding ambulatory appendages; 2–8, hæmal nerves from the brain. The nerves 7 and 8 carry the inhibitory fibers to the heart. 9–13, hæmal nerves from the thoracic ganglia, carrying accelerator fibers to the heart.

in the physiological behavior of these parts—for these structural differences do certainly exist.

The work of Carlson and of Garrey upon the heart of Limulus has thrown a new light upon the matter. Carlson has shown that in this form the cardiac contractions are purely of nervous origin. This result was the

more readily obtained because of the ease of separation of the nervous centers from the muscular portion of the heart (Figs. 32, 33, and 33*A*). There might almost be an excuse for thinking that Limulus was evolved for the purposes of laboratory investigation. Extirpation of the nerve cord causes an immediate and permanent stoppage of the normal rhythmic activity of the heart. That the muscle is still able to contract may be shown if it is stimulated by pinching or by an electrical current, when it will give a single contraction for each stimulus. The fact that the heart beats normally in a rhythmic manner must be due to the rhythmical activity of the nerve cells of the elongated heart ganglion. Carlson also found that the degree of automotism of the different regions of the heart of Limulus varied with the number of ganglion cells connected with them. As in the vertebrate heart, these cells are most numerous at the posterior or sinus end and least numerous at the anterior or aortic end of the heart.

To this evidence Garrey has added an accumulation of observations which make certain the neurogenic origin of the heart beat as far as this form is concerned. He shows that the temperature coefficient of the activity of the whole heart of Limulus for a 10° interval is the same as that obtained when the ganglion alone is subject to the temperature change. Further, he has shown that this temperature coefficient is that of chemical changes taking place within the ganglion cells—and, indeed, that the rate of the cardiac rhythm and the rate of carbon dioxide production by the ganglion cells run parallel courses; and still further, he has shown that stimulation of the inhibitory nerves of the heart of Limulus actually decreases, in the ganglion cells controlling the heart, those chemical processes which result in the production of carbon dioxide.

To Howell it seems doubtful whether these results of the work of Carlson and of Garrey can be applied to vertebrate hearts. He calls attention to certain characteristics in which Crustacean heart muscle differs from that of vertebrates, namely:

1. It has no refractory period.
2. It can be tetanized.
3. It gives submaximal contractions.

The Pacemaker of the Heart.—The portion of the heart in which resides the greatest rhythmic power really dominates the rest of the heart musculature and may be referred to as the *pacemaker*. In the hearts of the frog and turtle it is easy to show that the natural rate of the beat of the sinus is more rapid than that of the auricle or ventricle. Placing a tight ligature about the heart at the level of the sinoauricular junction will cause the auricle and ventricle to stop beating, at least for a time. When the beat of the ventricle is resumed, it is at a slower rate than that of the sinus. It can also be shown that when the temperature of the sinus is altered, the rate of contraction of the whole heart is modified, while

alteration of the temperature of the ventricle alone does not have this effect.

It is uncertain as to what structure should be called the pacemaker among invertebrated animals. It seems evident in many cases that the beat begins at the posterior end of the structure and passes rapidly forward. In the earthworm, where there is no true heart, the beat of the dorsal blood vessel begins at the posterior end of the body and runs rapidly toward the anterior end. There is still much to be done in this connection in order to give a complete account of the origination of the heart beat.

Among the warm-blooded animals it has been found somewhat difficult to homologize the parts of the heart with those of the hearts of the cold-blooded animals. There are three structures to be considered:

1. Auriculo-ventricular node (node of Stanley Kent) located at the base of the auricular septum. This is a collection of primitive cells and fibers and continues downward as a bundle (bundle of His) to its interventricular septum.

2. Sino-auricular node, discovered by Keith and Flack, which is located in the wall of the auricle. This part corresponds to the sinus venosus of cold-blooded vertebrates. It is club-shaped and has no apparent connection with the auriculo-ventricular node.

3. Concentration point. The muscles of the auricle radiate in a fanshape from a point which lies just below the sinoauricular node. This is the concentration point.

The beat appears to originate in the walls of the auricles, and in the sinoauricular node. The wave may be propagated along the musculature of the auricle to the auriculo-ventricular node, or possibly by some more direct route than the auricle muscle.

CHAPTER XV

PHYSIOLOGY OF THE HEART *(Continued)*

The knowledge of the existence of the various nerve elements in the vertebrates dates back to the work of the Brothers Weber in 1845. This work naturally led to the investigation of the invertebrates to discover whether such structures exist also in these less specialized forms. As the result of these studies, the occurrence of nerves having functions similar to the cardioregulators of the vertebrates in the Annelida, Crustacea, Arachnida (Limulus), and Mollusca is known.

Cardioregulator Nerves in Invertebrates.—Observations upon the nervous control of the pulsating vessels in the worms Arenicola and Nereis were made by Carlson in 1907. Seven years later observations upon the nervous control of the dorsal blood vessel of the earthworm *Lumbricus agricola* were made by a student in the author's laboratory. In the earthworm, stimulation of the ventral nerve cord gave evidence of the existence of both acceleratory and inhibitory fibers. These accelerations and inhibitions were less pronounced than has been described in higher animal groups, and no indication was found as to whether the nervous elements responsible for these controlled effects were located in the walls of the blood vessels or in the ganglia of the ventral nerve chain. That nervous tissues do exist in the walls of the pulsating blood vessels of Nereis and Arenicola has been well shown by Carlson. He calls particular attention to the fact that in the walls of the blood vessels of the forms mentioned there are found the same tissues in the same fundamental associations as in the higher forms, *viz.*, muscle cells, ganglion cells, and nerve fibers, but he was not able to establish microscopically the existence of connections between these nerve elements and those of the ventral nerve cord.

Carlson found in Arenicola that stimulation of the ventral nerve cord usually inhibited the œsophageal hearts in diastole, and at the same time increased the rate and force of the contractions of the dorsal blood vessels. In the earthworm, both accelerations and inhibitions have been obtained, with the former more clearly in evidence. Carlson's experiments indicate that the isolated vessels of Nereis and Arenicola continue to beat rhythmically after removal from the body, hence the mechanism for the origination of the beat must be localized in the muscle wall. This fact has undoubtedly had much influence with those who contend for the myogenic theory of the origin of rhythmic heart action.

Gaskell found that the musculature of the vascular apparatus of the higher worms is under nervous control. He found that in each segment of the leech Hirudo a small branch of the anterior nerve goes to the wall of the lateral blood vessel, and that this nerve is made up of fibers arising from the cells located in the central segmental ganglion. Some of the cells of this same ganglion are chromaffin cells. He found also that the lateral vessels have a rhythmic activity of six to eight contractions per minute, and that the rhythmic movement continues even though the nervous connections are severed. Gaskell, therefore, believes that the power of rhythmic contraction is inherent in the muscular tissue of these vessels. Gaskell found that stimulation of the anterior segmental nerve while the posterior nerve is intact slows the rhythm; that stimulation of the anterior segmental nerve after the posterior nerve has been cut accelerates the rhythm of the blood vessel. He also found that stimulation of the posterior segmental nerve after the anterior nerve was cut has no effect; but that stimulation of the posterior nerve after the worm has been decapitated slows the rhythmic action of the blood vessels. It may then be stated that the anterior nerve is an accelerator; its action is abolished by ergotoxin: the posterior nerve is an inhibitor; its action is abolished by curare. The nerve supply to the walls of the blood vessels is thus a double one, and corresponds to the double supply of the vertebrate heart through the vagus and sympathetic nerves.

It is evident from what has been said that there exists in this lowly group of animals the same set of physiological problems for properly distributing blood and in originating and maintaining vascular contractions as are found in the higher groups—and apparently worked out somewhat according to the same general plan. Certainly the same sets of tissues are involved and the tissues have essentially the same properties as occur in the higher forms, but developed to a lesser degree.

Mollusca.—Budington found that individuals of *Venus mercenaria* exhibit a good deal of variation in rate and character of heart beat, depending upon physiological conditions. In animals which are exhausted there is great irregularity. The significant result of Budington's work in this connection is that he found that stimulation of the visceral ganglion causes arrest of the heart. The curve drawn by the beating heart of Venus, subjected to electrical stimulation of the visceral ganglion, is strikingly like the curve of vagus inhibition of the heart of a frog or of a turtle. Stimulation of nerves passing from the visceral ganglion to the heart gives results entirely comparable to vagus stimulation, *viz.*, long aftereffects of strong stimulation, and typical escape from weak stimulation.

The following symbols are used in the figures of the nervous systems of certain of the Mollusca and Arthropoda. These figures are all taken from Carlson's paper, "Physiology of the Invertebrate Heart."

Abbreviations

A, anterior artery.
AC, abdominal commissures.
AOS, aortic sinus.
AU, auricle.
BG, branchial ganglion.
CC, cerebral commissure.
CG, cerebral ganglion.
CPC, cerebropedal commissure.
CVC, cephalic vena cava.
CVCO, cerebrovisceral commissure.
GV, gill ventricle.
H, heart.
HG, hind gut.
LA, lateral arteries.
LN, lateral nerves.
LVN, left visceral nerve.
MNC, dorsomedian nerve cord.
OER, œsophageal nerve cord.
OS, ostia.
PA, posterior aorta.
PC, pedal cord.
PG, pedal ganglion.
PLG, plural ganglion.
PLC, pleurovisceral cord.
PN, pallial nerve.
PVG, pleurovisceral ganglion.
PS, posterior sinus.
R, rectum.
RCN, recurrent nerve.
RV, renal vein.
RVN, right visceral nerve.
SA, sternal artery.
SL, suspensory ligaments.
SV, systemic ventricle.
THG, thoracic ganglion.
V, ventricle.
VC, visceral commissure.
VG, visceral ganglion.
VN, visceral nerve.

Carlson has shown that the auricles and ventricles of the Lamellibranchs, Mytilus (Fig. 34), Mya (Fig. 35), Tapes, Venus, and Pecten are supplied with inhibitory nerves from the visceral ganglia. The hearts of the Chitons (Fig. 36, Cryptochiton) are provided with accelerator nerves, reaching the heart from the palliovisceral nerve cords. In the Prosobranes (Fig. 37, Haliotis; Fig. 38, Sycotypus), Abalone, and the Limpet, stimulation of the œsophageal nervous complex or of the palliovisceral connectives, all the visceral organs being intact, or stimulation of the commissure in preparations in which a portion of the gills and the major part of the visceral hump has been removed has accelerator affects on both the auricles and the ventricle; if the heart is quiescent, the stimulation produces a series of beats; if in a state of rhythmical contraction, the rhythm is augmented.

Among the Tectibranchs (Fig. 39, Aplysia), cardioaccelerators are probably present in all forms, but inhibitory nerves have not been described. Among the Nudibranchs both acceleratory (possibly combined with augmentory) fibers and inhibitory fibers are found.

In the Pulmonate, Ariolimax (Fig. 40, Ariolimax), the evidence shows that the auricle is provided with both augmentor and inhibitor nerves; the ventricle is similarly provided with augmentor, and in all probability with inhibitor fibers, but the influence of the augmentor nerves is predominant. In Limax the auricle is provided with both augmentor and inhibitor nerves, and the ventricle is provided with inhibitory nerves which enter through the wall of the auricle.

In Helix, Carlson found that the auricle and ventricle are supplied with both accelerator fibers from the visceral nerve. Inhibitor and accelerator fibers enter the ventricle at the aortic end, and some inhibitor fibers reach the ventricle through the auricle. The nerves to the auricle enter at the base of that organ. The inhibitor fibers are the more readily excited, and their influence is the more marked.

The vascular system of the Cephalopods (Fig. 41, Loligo) is structurally much more complicated than that of any other of the Mollusca.

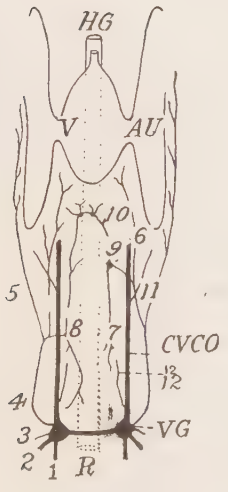


Fig. 34.

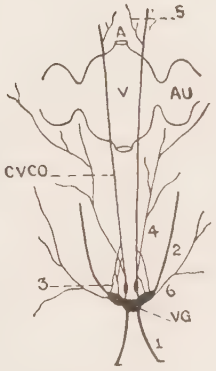


Fig. 35.

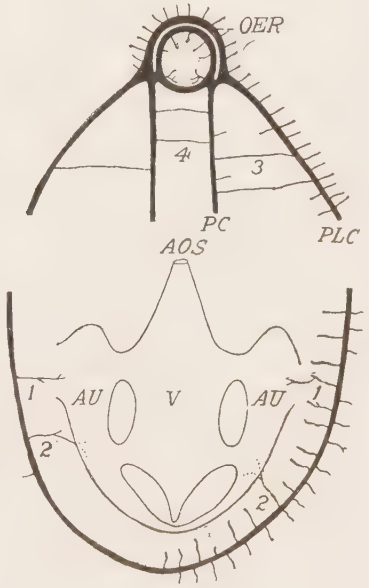


Fig. 36.

Fig. 34.—*Mytilus californianus.* Dorsal view. 1, posterior mantle nerve; 2, branchial nerve; 3, nerve to posterior adductor muscle; 4, nerves to dorsal body wall and pericardium; 5, nerves to base of auricles; 6, nerves to body wall and pericardium; 7, nerves passing posteriorly in the dorsal body wall; 8, 9, 10, ganglia on nerves 4; 11, nerves from the cerebrovisceral commissure to the kidneys; 12, nerves from the cerebrovisceral commissures to the adductor muscles of the foot and the byssus.

Fig. 35.—*Mya arenaria.* Ventral view. 1, nerve to mantle and siphon; 2, branchial nerve; 3, nerve plexus on the ventral surface of the kidney between the cerebrovisceral commissures and the visceral ganglion; 4, nerves to kidney (and heart); 5, nerves from the cerebrovisceral commissure to the reproductive glands; 6, nerves to kidney.

Fig. 36.—*Cryptochiton stelleri.* Ventral view. 1, nerves from pleurovisceral cords to auricles; 2, nerves that can be followed to the point of attachment of the auricles to the body wall; 3, 4, connectives between the nerve cords.

It is not at all surprising, therefore, to find that different investigators have reached diverging conclusions as the results of their investigations upon the cardioregulator nerves in this group. The study of the cardioregulators in these forms dates back to the investigations of Paul Bert, 1867, who was the first to discover the inhibitory influence upon the heart of the stimulation of the visceral nerve of a Cephalopod by means of

induced currents. Fredericq, 1878, found accelerator fibers going to the systemic heart of Octopus. Reinvestigations by Fuchs, Botazzi and Enriques, Carlson and Fry left a somewhat uncertain and confused state of affairs. Carlson concludes that the systemic and branchial hearts of the Cephalopods are supplied with inhibitory fibers from the two visceral commissures and there is some evidence that augmentor fibers reach the hearts from the same source. The rhythmically con-

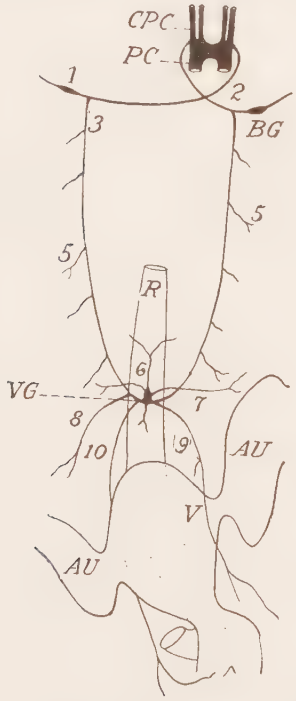


Fig. 37.

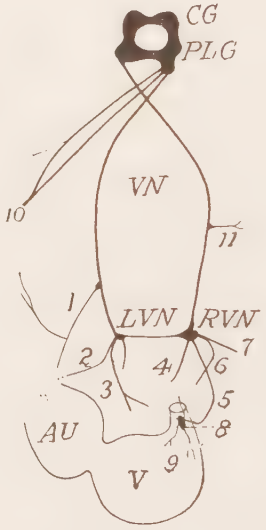


Fig. 38.

Fig. 37.—*Haliotis cracherodii.* Dorsal view. 1, 2, pleurovisceral commissure; 3, 4, nerves to the visceral ganglia; 5, nerves to floor of pallial cavity and efferent gill sinus; 6, nerve to rectum; 7, 8, nerves to base of auricles; 9, nerve to dorsal pericardium; 10, nerve to ventral wall of pericardium and aorta.

Fig. 38.—Sycotypus canaliculatus. Dorsal view. 1, nerve to floor of pallial cavity; 2, nerve to pericardium and auricle; 3, nerve to ventricular ganglion; 4, nerve to liver and genital organs; 5, nerve to ventricular ganglion; 7, renal nerve; 8, ganglion on ventriculo-aortic junction; 9, ventricular nerves; 10, pleurobranchial commissure; 11, nerve to hind gut.

tracting parts of the renal veins are in all probability supplied with inhibitory fibers from the visceral commissures.

Arthropoda.—In Palinurus (Fig. 42) it was found that stimulation of the thoracic ganglion with weak, interrupted currents resulted in inhibition of the heart, while the stimulation of the same ganglion with strong, interrupted currents resulted in an acceleration of the heart. It was also

found that stimulation of the small nerves arising from the dorsal side of the thoracic ganglion near the nerves going to the maxillipeds result in an inhibition, while the stimulation of the nerves arising from the dorsal side of the ganglion near the nerves going to the first ambulatory appendages result in an acceleration of the heart. From this it appears that there are both inhibitors and accelerators present and that they do not have the same threshold value for stimulation.

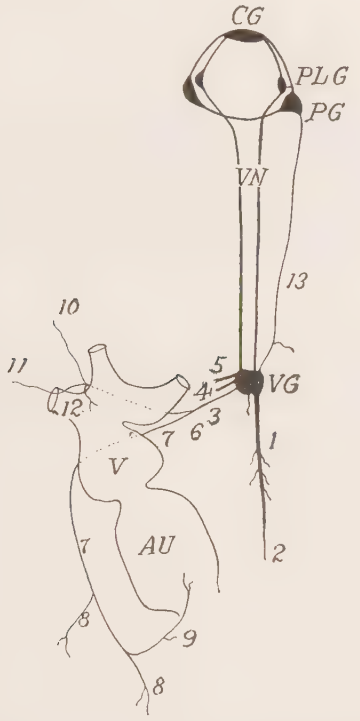


Fig. 39.

Fig. 40.

Fig. 39.—*Aplysia californica*. Dorsal view. 1, nerve to osphradium; 2, branch to roof of gill chamber; 3, nerve to viscera; 4, nerve to gill; 5, nerve to dorsum of the anal region; 6, nerve to liver, ventricle, and reproductive glands; 7, nerve to pericardium, kidney, and auricle; 8, branches to kidney; 9, branch to auricle; 10, branch to liver; 11, branch to the reproductive gland; 12, branch to aortic sinus; 13, nerve connecting right pedal and visceral ganglia.

Fig. 40.—*Ariolimax columbianus*. Dorsal view. 1, 2, 3, nerves to mantle and pallial cavity; 5, renocardiac nerve; 6, nerve to intestine and liver.

Similarly, Carlson has made brief studies of the conditions occurring in the tarantula, the Polyphemus moth, and the grasshopper, and has decided that each of these forms is provided with both cardioinhibitor and cardioaccelerator or augmentor nerves, and that these in the moth and grasshopper are given off not by the brain, but by the thoracic and possibly by the abdominal chain of ganglia.

Cardioregulator Nerves of the Vertebrates. 1. *Inhibitor Nerves.*—Among the reptiles and amphibia, inhibitory fibers pass to the heart from the central nervous system in the vagus nerve. Cutting the nerve causes the heart to beat more rapidly and results in increased blood pressure.

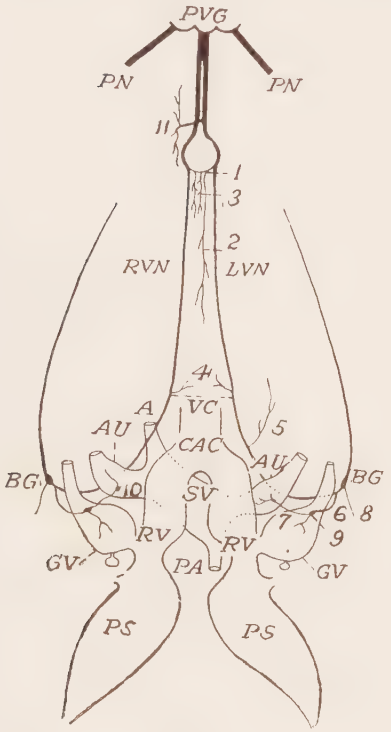


Fig. 41.

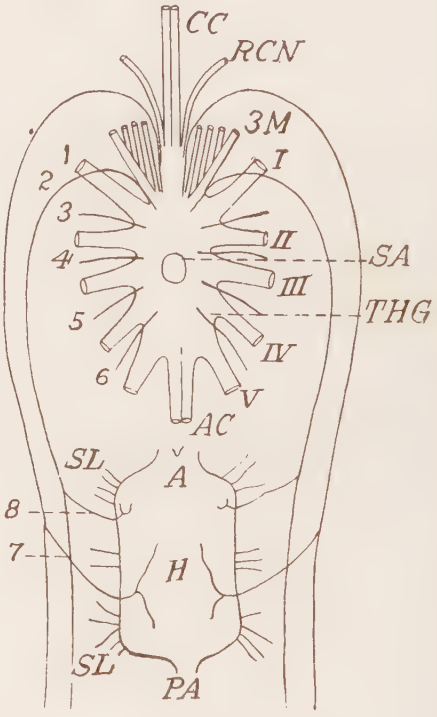


Fig. 42.

Fig. 41.—*Loligo pealii.* Ventral view. 1, commissure between the visceral nerves ventral to the vena cava; 2, nerve to the viscero-pericardial envelop; 3, nerves to rectum and duct of the ink gland; 4, nerves to ink gland; 5, nerve to penis; 6, cardiac nerve; 7, auricular nerves; 8, nerves to adductor muscles on the gills; 9, ganglia on gill ventricles; 10, ganglia on auricles; 11, nerves to vena cava.

Fig. 42.—*Palinurus* sp. Dorsal view, heart displaced posteriorly. I–V, nerves to corresponding ambulatory appendages; 3*m*, nerve to third maxilliped; 1, 2, nerves to adductor muscles and heart; 3–6, nerves ramifying in the arterial plexus on the adductor muscles; 7, 8, cardiac nerves.

Stimulation of the vagus	Auricular beat	Ventricular beat
With very weak current	Slows or weakens	No change
With stronger current	Slower and weaker	May strengthen
With strong current	Stops—and may finally escape	Stops—and may finally escape

Stimulation of the vagus appears to affect those parts of the heart in which rhythmicity is more highly developed (see Fig. 43).

2. *Accelerator and Augmentor Nerves.*—The heart of the frog is also under control of certain accelerator and augmentor fibers arising probably in the third sympathetic ganglion. Stimulation of the sympathetic nerve by means of a weak interrupted current accelerates and augments the beats after a rather long latent period. The effect here is antagonistic to that brought about by stimulation of the vagus nerve. Similar effects are obtained in the hearts of reptiles and mammals. In terms of metabolism, it may be stated that stimulation of the vagus by reducing the

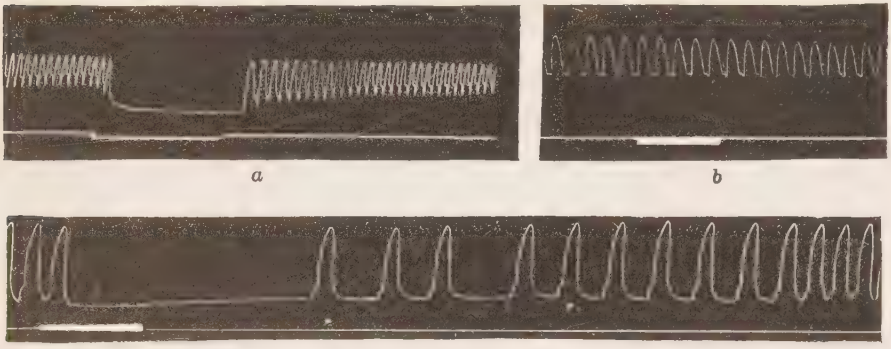


FIG. 43.—(Records read from left to right.) *a*. Record of normally beating frog heart subjected to vagus stimulation. Note that the heart contracts three times after the vagus is stimulated and that the inhibition continues after the stimulus is removed. *b*. Vagus stimulation of the same heart after the sinoauricular node had been painted with nicotine solution. *c*. Vagus inhibition of the heart of a turtle.

heart beat favors anabolism, while stimulation of the sympathetic by increasing the heart beat favors catabolism of the heart muscle. The aftereffect of vagus excitation is increased energy of contraction, while that of the sympathetic is exactly the opposite.

Heat Production by Heart Muscle.—Snyder has investigated the heat production by the heart muscle of the terrapin, and has found that heat production during spontaneous contraction begins with, or shortly after, the beginning of shortening of the muscle, reaches its greatest activity of output at or just before the height of contraction, and then continues in lessening amount during diastole for a period of time equal to about one-half of the time of the preceding contraction. The amount of heat liberated during the contraction of the terrapin ventricle has been shown by Snyder to be 0.155 calorie per gram per hour when beating at the rate of 5.4 per minute. This is the equivalent of the oxidation of 0.042 milligram of dextrose. Vernon has made measurements upon the CO_2 production of the tortoise heart which are in good harmony with this. In a heart beating at the rate of 5.5 per minute the output of CO_2

is from 41 to 47 cubic centimeters per hour per kilogram of heart. This is equivalent to about 0.054 milligram dextrose and 0.187 calorie heat per gram per hour.

The Law of the Heart.—E. H. Starling has made a very careful study of the mammalian heart under varying conditions and demands. Variations in bodily activity lead to great variations in the demands upon the transport system of the body. The heart must, under a great variety of conditions, maintain a head of pressure sufficient to drive the blood through the arterioles and capillaries and back to the heart. This calls for an unusual degree of adaptability to existing conditions, whether they be normal or pathological in character. Such adaptations may occur even after the nerves connecting the heart with the central nervous system have been destroyed. The governing mechanism must, therefore, be located in the heart walls—and very likely in the muscle fibers themselves.

The heart of a heart-lung preparation, provided the rate of inflow remains constant, forces on an almost perfectly constant amount of blood no matter whether the aortic pressure be high or low (within reasonable limits). Associated with this is the fact that as the arteriole pressure increases, the amount of blood passed through the coronary circulation increases, thus supplying the necessary increase in oxygen and food supplies which would be demanded by a muscle during extra work.

On the other hand, when the rate of inflow is varied, there is a very marked effect upon the rate of outflow. There is no damming up of the blood on the venous side, nor is there any failure of the heart to send on the blood from the venous to the arteriole side. The coronary supply is here not particularly modified. The muscular walls of the heart adjust themselves through the stretching of their fibers to the amount of blood sent in from the great veins.

The heart has also the power of adjusting its total energy changes to the work imposed upon it by the mechanical conditions under which it is working. As the demands upon the heart become greater there is an automatic increase in the energy expenditure necessary to meet the increased demand. A study of the changes in the volume of the ventricles and of the pressure inside the ventricles reveals the fact that "within physiological limits, the larger the volume of the heart the greater are the energy of its contraction and the amount of chemical change at each contraction." Starling has been able to show that the energy of contraction, however, measured as heat or as mechanical tension is a function of the length of the muscle fiber, as has also been shown by Hill to be true in the case of skeletal muscle.

The muscle substance, colloidal in its nature, is composed of unlike phases, arranged in longitudinal series, so as to give the appearance of fibrils. While there is no absolute proof of the exact conditions existing

in the fiber there is some ground for belief that the particles of the dispersed phase may be in the form of globules, and that these globules may, through the stretching of the fibrils during rest, become changed from a spherical to an ellipsoidal form, thus increasing the extent of the surface or interface between the two phases of the colloid. The rapidity with which the chemical energy of the muscle is converted into mechanical energy would seem to indicate that the fundamental changes must take place within almost molecular dimensions, and be at once translated into a change of tension. Under the influence of a stimulus there occurs in the muscle substance a chemical change which is accompanied by an electrical change. This electrical change may be assumed to occur at the surfaces or interfaces between the colloidal phases. Surface tension is the form in which surface energy is chiefly manifested. During periods of rest the surfaces between the phases have been at a low tension. With excitation of the muscle there occurs an electrical change, with the result that globules become electrically charged and also have their surface tension increased. The globules then tend to assume the spherical instead of the ellipsoidal form which had been assumed under the stretching of the fiber.

CHAPTER XVI

RESPIRATION

The term *respiration* involves the gas exchange which constantly takes place between an animal and its environment, that is, primarily, the intake of oxygen and the giving off of carbon dioxide but including also any other gas exchange which may occur.

The process is ordinarily separated into two groups of phenomena: (1) *external respiration*, that gas exchange taking place between the organism as a whole and the medium in which it lives; and (2) *internal respiration*, the exchange taking place between the cells of the animal body and the medium which is the immediate cellular environment. It will be noted that as one goes back farther and farther along the line of animal descent these two subdivisions of the respiratory process come closer and closer together, until in the protozoa and some of the lower metazoa the two processes are identical.

The gas interchange is of interest not alone as an isolated fact of common life, but for the significance which it has in making clear the nature of the fundamental chemical processes going on at all times in the animal body. From a study of the gas exchange it is possible to obtain information as to the carbon equilibrium of the body, the nature of the substances being broken down in the body, and a fairly accurate measurement of the total metabolism of the body during a given period.

The Need for Oxygen.—It is a matter of common experience that life processes of the higher animals cannot continue unless there is a continuous and abundant supply of oxygen. The same is true in the lower animals. Many of the chemical processes taking place in a living organism are oxidations. If these are to continue, a continuous oxygen supply is essential. The maintenance of protoplasmic structure is conditioned by a supply of oxygen. If the oxygen supply of even one of the simplest and lowest of animals forms is cut off or considerably reduced there is very quickly a stoppage of the ordinary life processes, and an actual disorganization of the material of which the organism is composed. This can readily be observed if one subjects such a form as Paramœcium or Amœba to conditions where the amount of oxygen is very greatly reduced. The animals come to move more slowly than is their habit, the protoplasm becomes filled with vacuoles which appear to contain a watery fluid, vesicles of liquid are protruded from the surface of the animal; these finally burst, the cell membrane breaks down, and the

protoplasm is extruded into the surrounding water. In certain forms it appears that the protoplasm of the animal is not all equally sensitive to the changes in oxygen content of the water. Certain portions of the cell disintegrate more rapidly than the others. The conditions induced by the absence of oxygen are very similar to those induced by poisoning with dilute solutions of potassium cyanide.

Historical Statement.—Our modern understanding of respiration dates back to John Mayo (1645-1679). He made very pertinent suggestions in 1668 concerning the analogy between respiration and combustion. Robert Hooke had demonstrated by experimental methods in the preceding year the need of all higher animals for a continual supply of air. Priestley (1733-1804) discovered oxygen and recognized its importance for all living matter. The present conception of respiration and nutrition is based directly upon the work of Lavoisier (1743-1794). He was the first to make a scientific quantitative study of respiration and to state that "life is a chemical action." He was the first definitely to recognize that animal heat is the result of the oxidation of body substance. With La Place he made numerous experiments upon respiration and animal heat.

Following these men, and deriving their inspiration directly from them, are Liebig, Regnault and Riset, Voit, Rubner, Pettenkofer, Atwater, Rosa, Armsby, and many others. Lavoisier determined a few of the fundamental facts of respiration. His experiments were upon man, and naturally subject to certain limitations. He found that the resting man uses far less oxygen than the man at work, that the consumption of oxygen is greater after the ingestion of food than before, and that lowered external temperature leads to greater use of oxygen.

It was then found that the blood holds large amounts of oxygen and carbon dioxide, and the suggestion was made that the blood was the seat of body oxidations. Liebig found that it was not elementary carbon and hydrogen which were consumed in the body, but proteins, carbohydrates, and fats. The combustion of these substances in the body is a part of the work included under the term *metabolism*. In this connection it should be pointed out that the presence of oxygen is not the cause of metabolic change. The combination of oxygen with tissue substance is due to the instability and activity of the substances making up living matter, and is accomplished, in part at least, through the agency of definite substances known as oxidases. The earlier experiments showed that atmospheric nitrogen is not used by the human machine, and also indicated that nitrogenous matters are not thrown off from either respiratory organs or skin in significant amounts, thus making it possible to make use of the nitrogenous content of urine and fæces to determine protein metabolism.

It was also shown by Pettenkofer and Voit that the amount of oxygen needed in the metabolism of the body depends upon the character of the substances oxidized. It is now known that:

100 grams of meat protein requires.....	133.43	grams of oxygen
100 grams of fat requires.....	288.5	grams of oxygen
100 grams of starch requires.....	118.5	grams of oxygen

It is just as true that the relation between oxygen consumed and carbon dioxide given off depends upon the character of the food substance oxidized.

It is common to refer to the ratio of the volume of carbon dioxide given off to the volume of oxygen absorbed as the respiratory quotient, and to express it in the form R. Q. or $\frac{CO_2}{O_2}$. If carbohydrate food alone is being consumed in the animal body, R. Q. = 1; if protein is being burned, R. Q. = 0.781; if fat is burned, R. Q. = 0.71. Upon a mixed diet including carbohydrates there is an increased output of carbon dioxide, and the R. Q. indicates that carbohydrates are being consumed. Lusk quotes a paragraph from Voit's obituary of Pettenkofer which may be included here.

> Imagine our sensations as the picture of the remarkable processes of the metabolism unrolled before our eyes, and a mass of new facts became known to us! We found that in starvation protein and fat alone were burned, that during work more fat was burned, and that less fat was consumed during rest, especially during sleep; that the carnivorous dog could maintain himself on an exclusive protein diet, and if to such a protein diet fat were added, the fat was almost entirely deposited in the body; that carbohydrates, on the contrary, were burned no matter how much was given and that they, like the fat of the food, protected the body from fat loss, although more carbohydrates than fat had to be given to effect this purpose; that the metabolism in the body was not proportional to the combustibility of the substances outside the body, but that protein, which burns with difficulty outside, metabolizes with the greatest ease, then carbohydrates, while fat, which readily burns outside, is the most difficultly combustible in the organism.

During succeeding investigations certain general facts have been discovered. Among these are the following:

1. Voit found that protein metabolism is not increased by muscular activity.

2. Metabolism is not proportional to oxygen supply (in the higher animals).

3. Absorption of oxygen is not the cause of metabolism. On the contrary, the rate of metabolism determines the amount of oxygen to be absorbed.

4. Respiration is regulated through oxygen requirement, and carbon dioxide production. This is effected through variation in oxygen and carbon dioxide content of the blood stream.

The metabolic work of the body calls for the expenditure of energy. This energy must come from some source. The effect of sunlight upon carbon dioxide and water is to form formic aldehyde, which may then be built up into sugar, with the giving off of oxygen. The energy of the sunlight is stored in the sugar or starch and will be set free when these are broken down again into carbon dioxide and water. Considerable study has been given to the relative values of different foods, and to the determination of the degree to which one may be substituted for another. As a result, it is stated that foodstuffs may be substituted for each other (under given conditions) in accordance with their heat-producing value. Leaving out of consideration the need of nitrogenous food for the upbuilding of protoplasm, Rubner states that 100 grams fat, 232 grams starch, 234 grams cane sugar, and 243 grams dried meat have the same fuel value (are isodynamic).

Organs of Respiration.—There is much variation in the character of the respiratory organs found among animals as well as in the degree of development of the various types of structures found. It will be readily understood that, where an animal has a small mass of living substance as compared with the total of body surface, it is much easier for the animal to carry on a sufficient gas exchange with its surroundings than is true for an animal which has a much larger bulk of body tissue in proportion to the usable respiratory surface. Also, different groups of animals have found it advantageous to make use of different types of respiratory organs because of the different environmental conditions to which they are exposed.

Fundamentally, any respiratory organ is simply a modification of body surface through ingrowth or outgrowth so as to provide a means for bringing the body fluid or body cells into close relationship with the oxygen supply of air or water. In a very large number of forms the respiratory and circulatory systems work together in carrying on this function. In one very large group of animals, the insects, there appears little, if any, respiratory work done by the blood or the circulatory system. In this case the oxygen supply is brought close to the tissue cells of the body by means of definite air passages which ramify everywhere throughout the body mass.

The following types of respiratory structures may be recognized:

1. *General body surface*, the organ for respiration in Protozoa, Cœlenterates, and worms.

2. *Respiratory papillæ*, evaginations from the cœlom in the form of finger-shaped tubules, filled with the cœlomic fluid of the animal and surrounded by sea water.

3. *Respiratory tree*, a tubular, frequently much-branched outgrowth from the alimentary canal in such forms as the starfish, Echinoids, and Holothurians. This tubule is intermittently filled with sea water and emptied through the cloacal opening.

4. *Gills*, filamentous or plate-like appendages in which the blood circulates, separated from the surrounding water only by thin membranes, through which the oxygen dissolved in the water is absorbed and the waste products are given off. In the water-breathing vertebrates, as the fishes, the gills are situated at the side of the pharynx and the water taken in through the mouth passes out through the branchial clefts, bathing the gills which are attached to the arches separating the clefts. In the invertebrates the gills are variously located, the exact position depending upon the life habit of the animal.

5. *Tracheæ*, air-conveying tubules forming the respiratory system of most insects, myriapods, arachnids, and the protracheata. In the true insects they form a system of ramifying and anastomosing tubules, enlarged at certain points into air sacs, and penetrating to nearly all parts of the body. Their external openings, the stigmata, or spiracles, are located along the sides of the body, and are often provided with valves. The tracheæ have a chitinous lining which is stiffened by a spiral fiber or fiber-like thickening.

6. *Lungs*, sometimes simple but more frequently extremely compound, sacular structures connecting with the anterior portion of the alimentary tract through the trachea. From a physiological point of view, the fundamentally important portions of the lung are the air sacs in which the inspired air comes into immediate contact with a moist, mucous membrane richly supplied with blood capillaries through which oxygen may diffuse from the air sac into the blood and carbon dioxide from the blood into the air sacs. The form, size, and degree of development of lung structures vary very much in the different animal groups.

It will be well to point out here that as far as the regulation of breathing is concerned there is no provision for breathing movements so long as the surface of the body is sufficient to supply the need of oxygen, as, for example, in embryos and larvæ of amphibians. With increased need of oxygen there is an increased respiratory surface, and with this increase in surface provision is made for regulating the supply of water or air coming into contact with the surface. A somewhat similar adaptation is found in the red blood cells, where it appears that those forms whose size and life habit demand the least oxygen have the largest erythrocytes, that is, the least surface for taking up oxygen; those whose life habit demands a greater supply of oxygen have smaller cells with a total greater surface for oxygen absorption.

The Gas Laws.—In any consideration of respiration the so-called gas laws must be kept in mind. The statements of these laws have been

recently revised by Haldane as the result of extended investigation, and in the revised forms are stated as follows:

Boyle's Law.—The intermolecular volume of a gas varies inversely as the pressure, when the volume and temperature are constant.

Charles's Law.—At constant pressure the intermolecular volume of a gas varies directly as the absolute temperature.

Avogadro's Law.—The number of molecules in a given volume of any gas at the same temperature and pressure varies as the intermolecular pressure.

Or the three laws may be combined into the following statement: The intermolecular volume (that is, the proportion of free space) of a given volume of gas or liquid varies inversely as its mean, intermolecular pressure, and directly as the absolute temperature, the concentration of gram molecules, and a constant which is the same for all gases and liquids.

To these should be added *Henry's Law:* The quantity of a gas (either weight or volume at normal temperature and pressure) dissolved by a given liquid at a given temperature is directly proportional to the pressure under which the absorption takes place; if, for example, the pressure on the gas is doubled, twice as much of it will be forced into solution.

Also the law of partial pressures must be kept in mind: In a mixture of gases each gas exerts the same pressure that it would exert if it alone occupied the space.

The Tension of a Gas.—In the work upon respiration there will be occasion frequently to refer to the amount of gas available in a given solution or a given position in the animal body. In designating the concentration of the gas, it is customary to make use of the words *tension* or *partial pressure.* It is understood that oxygen comprises about 21 per cent of atmospheric air, and that the normal barometric pressure at sea level is 760 millimeters of mercury. This means, according to the law of partial pressures, that 21 per cent of the 760 millimeters of mercury pressure is exerted by the oxygen of the atmosphere and the remainder by the other gases in the atmosphere. So, when speaking of the tension of oxygen as being 2 per cent, 2 per cent of an atmosphere is meant, or 2 per cent of 760 millimeters of mercury pressure.

Absorption of a Gas by a Liquid.—The definite relationship which exists between a gas and a liquid by which it is absorbed may be expressed by means of the *absorption coefficient.* This is defined as the volume of a gas which is absorbed by a unit volume of the liquid under normal pressure (that is, 1 atmosphere). The statement that the absorption coefficient of oxygen in water at 20° C. is 0.031 means that 1 cubic centimeter of water absorbs, under 1 atmosphere of pressure, 0.031 cubic centimeter of oxygen measured at 0° Celsius and 760 millimeters pressure.

TABLE 32.—TABLE GIVING THE WATER-ABSORPTION COEFFICIENTS OF OXYGEN, NITROGEN, AND CARBON DIOXIDE AT DIFFERENT TEMPERATURES

Temperature	Oxygen	Nitrogen	Carbon dioxide
0	0.0489	0.0239	1.713
10	0.0380	0.0196	1.194
20	0.0310	0.0164	0.878
30	0.0262	0.0138	0.665
40	0.0231	0.0118	0.530

The facts presented in the table would indicate that in ordinary air at 20°C., composed approximately of 79 per cent of nitrogen and 21 per cent of oxygen, there might be absorbed by water standing an indefinite time, and undisturbed by outside forces, 21 per cent of 31 cubic centimeters per liter, or 6.5 cubic centimeters per liter. It is to be noted that the amount of oxygen absorbed by the water decreases steadily as the temperature of the water rises, so that at 30° the water absorbs only a little more than half as much oxygen as it does at 0°. The significance of this fact for animal life will be pointed out in another connection.

Water as a Respiratory Medium.—It is important for the respiratory processes of aquatic animals that the oxygen supply should be unfailing. It appears that most animals have little power to store up any oxygen against a time of need. The table given above represents the absorptive power of pure water for oxygen, carbon dioxide, and nitrogen under laboratory conditions. It is important to know whether the waters of oceans, lakes, and streams actually contain oxygen in any such proportions as suggested in the table. Many investigations have been made to determine the concentration of dissolved gases. Some of the results are shown in the following table.

TABLE 33.—TABLE SHOWING AMOUNT OF DISSOLVED GASES IN CUBIC CENTIMETERS PER LITER

	Oxygen, cubic centimeters per liter	Nitrogen, cubic centimeters per liter	Free and combined carbon dioxide, cubic centimeters per liter
Sea water (Jolyet and Regnard)	4.8–6.3	12.5 –14.1	43.6 –71.2
Sea water (Vernon)	5.7–5.8	12.88–12.91	46.88–46.91
Sea water (Naples Laboratory)	3.3–5.74	11.76–12.03	65.78–74.29
Seine River (Grehant)	6.0–8.0	13.0 –17.0	20.0 –30.0
Seine River	7.0–9.0	15.0	23.3

Birge and Juday have examined the waters of many of the lakes of Wisconsin and of New York. While there are many variations in the

oxygen content due to a variety of local conditions and to the temperature differences due to seasonal change, it may be said that the waters examined indicate very clearly that the ordinary laws of diffusion hold good for these lakes—but that diffusion must be assisted by currents and the spring and autumnal overturns in order to provide for equal distribution of oxygen through the water at all depths.

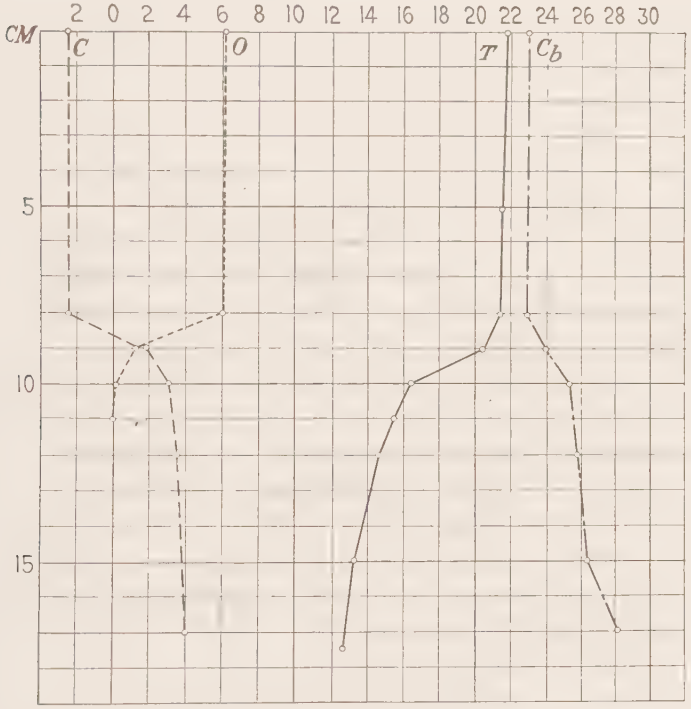


FIG. 44.—Conesus Lake, Aug. 25, 1910. The curves for dissolved gases are designated as follows: *C* = carbon dioxide; that portion to the left of the zero line indicates the alkalinity and that to the right the free carbon dioxide; *Cb* = fixed carbon dioxide; *O* = oxygen; and *T* = temperature. The vertical spaces represent the depth in meters and the horizontal spaces show the temperature in degrees Centigrade and the cubic centimeters of gas per liter of water at normal temperature and pressure. The depths at which observations were made are indicated by small circles and these points have been connected directly without any attempt to round off the curves. (*From Birge and Juday.*)

Distribution of Oxygen, Carbon Dioxide, and Temperature in Finger Lakes of Central New York.—In so far as distribution of oxygen is concerned, Birge and Juday find that in lakes of the temperate type, in which there is a complete autumnal and vernal overturning and circulation of the water, there is during the summer season a great variation in the concentrations of oxygen at different depths. They refer to three strata of water which (during the summer season) are more or less well defined.

Epilimnion.—This is the stratum at and just below the surface, in which the temperature is fairly uniform. This layer may have a maximum thickness of 12 to 15 meters before the temperature of the water begins to decline in the fall.

Thermocline.—The thermocline is that region of the lake lying immediately below the epilimnion, in which the temperature falls rapidly. The thickness of this layer is subject to much variation because of oscillations of the water.

Hypolimnion.—In this layer the temperature falls rapidly, then more slowly, the curve approaching a straight line. The lower part of very

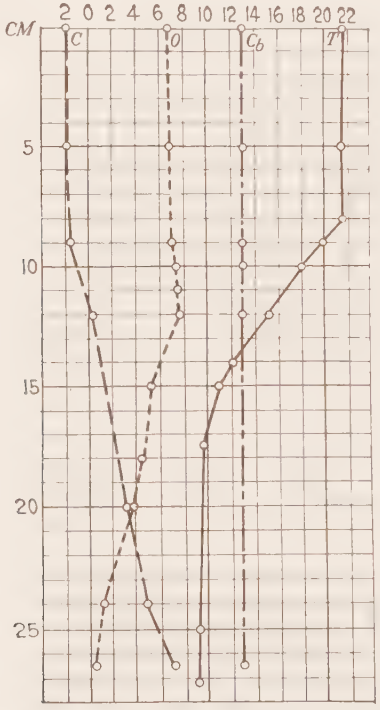


FIG. 45.—Dissolved gases, Hemlock Lake, Aug. 23, 1910. For explanation see Fig. 44. (*From Birge and Juday.*)

deep lakes may have a temperature nearly, or quite, the same through a considerable thickness of water.

Birge and Juday made gas analyses of the water of a large number of lakes in Wisconsin and New York in order to discover the distribution of the gases during the late summer. They found that different lakes show wide differences with respect to the quantity of dissolved oxygen in the hypolimnion, and that they fall into three groups:

1. Those in which some or practically all of the hypolimnion is devoid of free oxygen in the later stage of the stratification period.

2. Those in which there is a marked decrease of oxygen in some or all of the hypolimnion but not an actual disappearance of it.

3. Those in which the decrease of oxygen in this layer is comparatively slight.

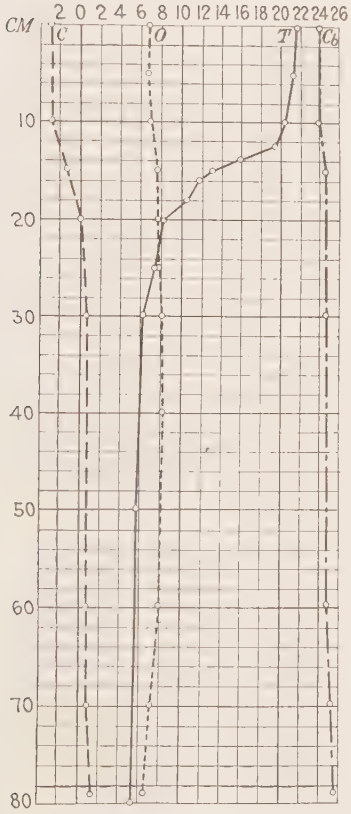


Fig. 46.

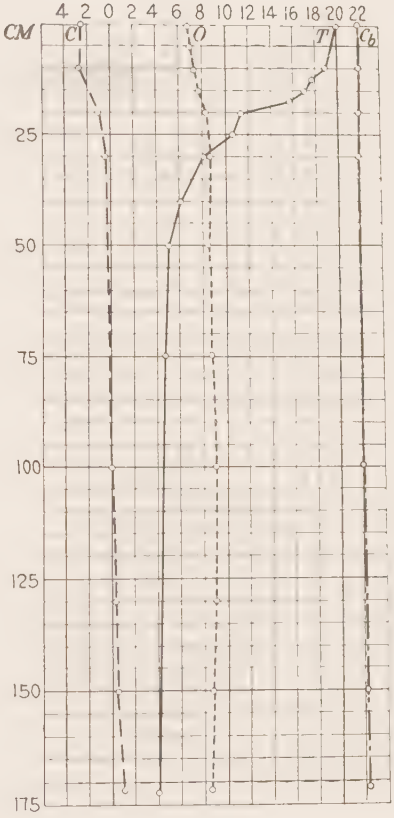


Fig 47.

Fig. 46.—Dissolved gases, Canandaigua Lake, Aug. 20, 1910. For explanation see Fig. 44. (*From Birge and Juday.*)

Fig. 47.—Dissolved gases, Seneca Lake, Aug. 4, 1910. For explanation see Fig. 44. (*From Birge and Juday.*)

In order to show more clearly the results of these investigators, and the significance of their results in as far as the respirations of animal life is concerned, the following charts are shown here. Figure 44 indicates their findings for Conesus Lake, N. Y., a lake of the first class. All of the water below 10 meters either possessed no oxygen at all or only small traces of it. Oxygen disappears from the bottom water because of decom-

position of plankton and shallow-water and shore vegetation. Figure 45 shows the findings for Hemlock Lake, a lake of the second class. Figures 46 and 47 show the findings for Canandaigua Lake and Seneca Lake, both lakes of the third class. In the two last lakes the amount of decomposable material is small—or the volume of the hypolimnion is relatively larger—so that there is less decrease in the oxygen content. This makes for better respiratory conditions for existing animal life.

Variations in Oxygen Content of Water under Special Conditions. — It has been found that the water of a river shows great variations from the normal in its passage through a city from which it receives large amounts of sewage and industrial wastes. The oxidation, by means of bacteria, of the organic matter thrown into the stream involves a very great reduction in the available supply of oxygen for other forms of organic life. The Thames River above the city of London has been shown to have an oxygen content of 7.4 cubic centimeters of oxygen per liter. At Woolwich the oxygen content has fallen to 0.25 cubic centimeter per liter. This amount of oxygen in the water will permit leeches and a few of the more resistant fishes to live. Crayfishes and other forms that require large amounts of oxygen cannot live in water of such low oxygen content.

It has been pointed out that water at 0° absorbs approximately twice as much oxygen as at 30°C. It is well known also that chemical operations go on much more rapidly at higher temperatures than at lower. It is also true that many of the chemical operations going on in the animal body are oxidations. It would, therefore, appear that with an increase in the body temperature of an aquatic animal there would be an increased rate in all of the chemical reactions going on within the body —and a reduced amount of oxygen available with which to carry on certain of these processes. The high temperature might easily become fatal simply through its enforced reduction in the oxygen supply. Of course, sight must not be lost of the fact that there are also other factors which might occasion fatal results following a rise in temperature, such as the coagulation of the colloids of the cells.

It is stated also that there are no fishes in the high-lying lakes of the Andes, due possibly to the fact that the oxygen content of the water under the reduced partial pressure of the oxygen of the atmosphere is so small as to make it impossible for such animals to secure sufficient oxygen for carrying on ordinary life processes. It is, of course, possible that the absence of fish from these lakes may be due to another cause. It has been shown also that the ill effects observed when fish are asphyxiated in water containing too little oxygen are due to the lack of oxygen and not to an increase of carbon dioxide. By the addition of oxygen fish have been found to live in water containing as much as 211 cubic centimeters of carbon dioxide per liter.

The great handicap under which the aquatic animal is living may be realized when it is considered that a liter of air at ordinary temperature contains 210 cubic centimeters of oxygen. A liter of water contains only from 3 to 9 cubic centimeters of oxygen. This means that the aquatic animals have at their disposal at the most in a liter of their immediate environment less than one-twentieth of the amount of oxygen which an air-breathing animal has. An animal which lives in the water is somewhat in the position of a land animal in whose air the oxygen content has sunk below 1 per cent.

Diffusion of oxygen from the air into water requires considerable time. It has been shown that if the water of the sea depended upon diffusion alone it would require several centuries for oxygen to diffuse from the surface to the bottom of the sea. This slowness of diffusion is a factor of importance in situations where the water contains numerous bacteria, and where decaying substances are using up a large amount of oxygen. It has been shown in certain cases that when such water is saturated by shaking with air it may lose one-half of its oxygen content within one-fourth of an hour.

It is also just as true that under certain conditions the water may come to be supersaturated with oxygen. Knauthe found that in the water of a certain pond which contained a large number of Euglena (a chlorophyll-bearing protozoan) there was an oxygen content of 22 cubic centimeters per liter while the normal amount at that temperature (15°) would have been 7.1 cubic centimeters per liter. This amount is certainly larger than pure water will absorb, even by shaking. Corresponding to the very large oxygen content of the water during the day it was found that at night there was a great oxygen reduction, down to 2 cubic centimeters per liter, due undoubtedly to the oxygen consumption of the animals living in the water. Under such conditions it can be well understood how in the presence of large numbers of bacteria or other oxygen consumers the oxygen content of the water can be reduced to a point which will mean asphyxiation for fishes or other forms having large oxygen needs.

The *value of chlorophyll* in keeping up the oxygen content of the water must be clearly apparent. That chlorophyll is an exceedingly sensitive substance may not be realized. The work of photosynthesis by green aquatic plants apparently goes on at all times when there is light. Even moonlight is sufficiently strong in its effects to cause chlorophyll to do its work. One pond showed an increase in contained oxygen from 2.7 to 4.6 cubic centimeters per liter in the course of 1½ hours of moonshine. During the winter season the activity of animal life is lessened and the oxygen consumption correspondingly reduced. On the other hand, the constructive work of the chlorophyll-bearing organisms is very lively. Much oxygen is being given off and little being used. By

cutting through transparent ice, samples of water have been secured which carried oxygen to the extent of 46 cubic centimeters per liter the water being almost saturated with pure oxygen. Under opaque ice the light is almost wholly shut off from chlorophyll-bearing organisms and the work of photosynthesis goes on at a much reduced rate or not at all. Under such conditions the oxygen content of the water becomes gradually diminished, even to such an extent that fishes may be asphyxiated. The practical value of cutting holes in the ice during the winter, as far as fish life is concerned, consists not in the admission of the air to the water, but the admission of light, which will serve to set into operation the photosynthetic processes of the green plants.

Air in the Depths of the Ocean.—Juday takes the position that diffusion alone must play a very unimportant part as far as the aeration of the depths of the ocean is concerned. Atmospheric gases diffuse very slowly through water. He calls attention to Hufner's computations for the Bodensee (a lake in Austria), which has a maximum depth of about 250 meters. His results show, first, that it would take oxygen about 42⅓ years to pass from the surface to the bottom of this lake by diffusion alone; second, that it would require more than 100,000 years for the quantity of oxygen which its waters at 10°C. are capable of holding to diffuse into a body of water of equal area and unlimited depth: third, under natural conditions with a depth limited to 250 meters it would require over a million years for this body of water to become saturated at the above temperature if it had no dissolved oxygen and acquired a supply only by diffusion from the atmosphere.

If ocean waters were aerated solely by diffusion from the atmosphere, we should expect the upper strata to possess a larger amount of dissolved oxygen than the lower. But such is not the case in the tropical Atlantic, for example. Here the smallest amounts, 1 to 2 cubic centimeters of oxygen per liter of water, are found between the depths of 150 and 800 meters, while the water between 1100 and 1500 meters contains twice as much or more, that is, 3 to 4 cubic centimeters per liter.

The Black Sea affords an excellent illustration of the inefficiency of diffusion in the process of aeration. Owing to greater salinity, hence greater density, of the lower water the vertical currents do not penetrate to the bottom of the sea; that is, the lower portion is permanently stagnant and oxygen can only pass into it by diffusion. Lebedinzeff found no dissolved oxygen below a depth of 200 meters, the aerated portion comprising only about 8 per cent of the maximum depth of this body of water.

Similar conditions may be found in many freshwater lakes during the summer period of thermal stratification. At this time the cool lower stratum of water is cut off from contact with the air by the warm upper stratum and can receive new supplies of oxygen only by diffusion from

the latter. If the lower stratum loses any or all of its dissolved oxygen during the stagnation period the deficiency continues until the autumnal overturning of the water.

In view of these facts, it is evident that some agent other than diffusion is responsible for the aeration of bodies of water. In lakes, aeration is accomplished by the vernal and autumnal overturning of the water and its subsequent circulation for a longer or shorter period. In speaking of the aeration of ocean waters, Helland-Hansen states that "those gases are absorbed at the surface from the atmosphere and are carried by currents even into the deepest parts of the ocean in varying amounts."

Aerobic and Anaerobic Respiration.—A distinction must be made between two classes of animal organisms as far as their respiratory processes are concerned. These are:

1. *Aerobic Organisms.*—Those which grow and thrive only in the presence of free oxygen.

2. *Anaerobic Organisms.*—Those able to live in the absence of free oxygen.

While numerous cases of anoxybiosis are known among the cold-blooded animals, there are no clear cases known among the warm-blooded animals. Pflüger kept frogs for as long as 24 hours in pure nitrogen, and found that the amount of carbon dioxide produced under such conditions was not very different from that produced by animals living in the air. These experiments are not entirely conclusive, because the animals kept in the nitrogen were on the whole more active than those kept in the air, and there is also the possibility that some of the carbon dioxide liberated during the stay in the nitrogen was preformed in the tissues of the frogs. On the other hand, it may be stated that the amount of carbon dioxide produced was so large that it is hardly to be doubted that some of it was produced in an anoxybiotic metabolism which may or may not be a stage in the normal oxidative breakdown of the nutritive substances.

The supposition has often been expressed that the source of the muscle energy is not found so much in the oxidative processes going on within the cells as in some of the other chemical or physical reactions which take place within them. The oxidative processes certainly do lead to heat production. It was this conception that lead Bunge to a consideration of the respiratory conditions among intestinal parasites.

Among the entozoa of warm-blooded animals the oxygen need is reduced to a minimum. In the alimentary tracts of some of the higher animals the most careful quantitative determinations have failed to indicate the presence of oxygen. In fact, powerful reduction processes may take place without hindrance. It might be thought that the parasites would be able to take their needed oxygen from the tissues, to which they so frequently attach themselves.

Bunge removed intestinal worms (Ascaris?) from the cat and from pike and placed them in normal saline solutions containing soda. The oxygen contained in the liquid had been previously removed by boiling and by exposure to strong reducing agents, such as pyrogallol. In this liquid, completely lacking in oxygen, the worms lived 4 to 6 days, during which time they carried on active movements and produced large quantities of carbon dioxide, which could only have come from the breaking down of substances in their tissues. When oxygen is offered to them they are unable to make use of it. The processes involved in this case have been studied by Weinland, with the result that it now seems probable that the carbohydrates were the substances catabolized by the worms, the process leading to the production of carbon dioxide and the fatty acids, valerianic and caproic. Weinland is of the opinion that glycogen is the source of the material broken down, since it forms about one-third of the dry substance of the worms.

Similarly, Lesser has found in the case of the earthworm that carbon dioxide and fatty acids are produced in the absence of oxygen. When air is admitted to earthworms after anoxybiotic period, there is observed an abnormally high intake of oxygen with a very low respiratory quotient.

The slime or mud-dwelling forms live under conditions somewhat similar to those common to the intestinal parasites. In the slime at the bottoms of ponds there are produced also quantities of carbon dioxide, marsh gas, and other substances of a noxious character. Animals taken from such habitats will live for short periods if placed in boiled water: crabs, water beetles, mites, and wood lice for a few hours, earthworms for 1 day, planarian worms from 1 to 2 days, horse leeches 3 days, and leeches 4 days.

It is possible also that the case of the so-called vinegar eel (*Anguillula aceti*) should be included here. Certain it is that these forms may live for a very considerable time under conditions of very much reduced oxygen pressure. While the experiments cannot at the present time be considered as satisfactorily conclusive, it appears that these forms may live for as long a time as 3 months in vinegar contained within a glass tube from which as much of the air as possible has been pumped by means of a water-jet pump, and the tube then sealed off in a flame. In this case the necessary oxygen for the carrying on of body processes must have come from the splitting of some compound in the vinegar.

It has been found also by Huss that the crustacean Cyclops sometimes exhibits a remarkable resistance to lack of oxygen and to the presence of substances usually considered toxic in character. Cyclops were found by this author living in a bay near Stockholm at a depth of 10 feet in water which contained up to 4.4 milligrams H_2S per liter. Experiments performed in the laboratory also indicated the possibility that these animals could live for 33 minutes in water free from oxygen and containing

70 milligrams H_2S per liter, and for 10 minutes in water containing 140 milligrams H_2S per liter.

The early observations of Newport may be mentioned here. He found that the larvæ of a butterfly would revive upon transfer to normal air after having become apparently dead due to a stay in a hydrogen atmosphere for 12 hours, or after 2 hours under water. These animals were very quickly killed by chlorine gas or hydrocyanic acid.

CHAPTER XVII

RESPIRATION (*Continued*)

Dr. Lund has developed a very simple technic for the determination of carbon dioxide in such forms as Paramœcium. A bottle of about 2 liters capacity is filled with 0.01 *N* HCl and a second 2-liter bottle with $Ba(OH)_2$ of any concentration between *N* 75 and *N* 150. Both bottles are provided with soda lime tubes and burettes. The solution should be made up of carbon-dioxide-free distilled water as described by Treadwell and Hall (1915). A bottle of about 100-cubic-centimeter capacity with an extra-large mouth is provided with an accurately fitting glass stopper. To the under side of the latter there is cemented a hook from which hangs a slender dish small enough to enter the mouth of the bottle. From 1 to 5 cubic centimeters of a suspension of Paramœcium, which have previously been gradually transferred to tap water from hay infusion, are placed in the dish; 10 to 15 cubic centimeters of the standard barium hydroxide solution are run into the bottle through a small hole in a cardboard disc placed over the mouth of the bottle, and the stopper with dish attached is quickly inserted. At the end of the desired period, the stopper and the dish are removed, the bottle is again covered with the cardboard disc, phenolphthalein is added, and the unabsorbed barium hydroxide titrated through the hole in the disc with the 0.01 *N* HCl. As a control, a similar bottle is set up in exactly the same way but without the animals. It is well to work before an open window through which fresh air is entering to sweep the carbon dioxide of the laboratory away from the titration, thus making the carbon dioxide of the air the same in duplicate bottles. The carbon dioxide in the bottle is determined by titrating 15 cubic centimeters standard barium hydroxide in each of the two bottles, one immediately, and the other at the end of 1 hour, the absorption of carbon dioxide under the conditions being complete in that time. The applicability of the method is shown by the fact that twice the number of Paramœcia always produce twice the amount of carbon dioxide. The method is simple, direct, rapid for a large number of determinations, and has a considerable range of adaptability and a small percentage of error.

Respiration in Protozoa.—Lund has discovered by means of a modified Winkler method the following facts regarding the use of oxygen by Paramœcium caudatum.

1. It does not vary with variations in the oxygen concentration of the water unless these variations are sufficient to cause injury or actually prevent metabolic processes. The concentration of oxygen may vary from a minimum of 0.02915 cubic centimeter oxygen, N.T.P. per 100 cubic centimeters water to 1.665 cubic centimeters at N.T.P. per 100 cubic centimeters, or fifty-five times the minimal concentration, without affecting the rate of oxidations, at temperatures of 13.5 and 25.0°C.

2. It is not influenced by KCN unless this substance occurs in an amount sufficient to cause cytolysis. Potassium cyanide does not inhibit oxidative processes which might take place after cytolysis has occurred, because oxidation stops when cytolysis has been brought about by other means, such as mechanical injury or too much oxygen.

3. It is very largely determined by the amount of nutritive material contained within the cell. The process of mitosis may be left out of consideration here since it does not take place during the period of experimentation.

The gas exchange of *Colpidium colpoda* was investigated by Thedor Wachendorff. The more important results of his work are included in Table 34.

Table 34.—Gas Exchange of Colpidium Colpoda

Experiment	Number of animals	Duration in hours	Oxygen, cubic centimeters	Carbon dioxide, cubic centimeters	$\frac{CO_2}{O_2}$	O_2 in milligrams per million animals	CO_2
1	*a.* 105,000–150,000..	22	0.461	0.172	0.37	0.273	0.140
	b. 105,000–150,000..	28	0.576	0.160	0.31	0.178	0.114
2	*a.* 140,000–170,000..	6.5	0.272	0.092	0.34	0.386	0.180
	b. 140,000–170,000..	23	0.554	0.202	0.36	0.222	0.112
3	*a.* 233,000–243,000..	17.5	0.192	0.046	0.24	0.0658	0.022
	b. 233,000–243,000..	41.5	0.296	0.072	0.25	0.0428	0.0143
	c. 233,000–243,000..	113	0.619	0.144	0.23	0.033	0.011
4	*a.* 160,000–180,000..	42	0.422	0.138	0.33	0.0845	0.0381
	b. 160,000–180,000..	73	0.392	0.155	0.39	0.0452	0.0246

High concentrations of oxygen are fatal to many species of protozoa parasitic in the intestines of other animals. It might well be thought that under their natural conditions they live in a situation in which there is an exceedingly limited supply of free oxygen, and that it is necessary for these protozoa to secure their oxygen by some anaerobic process. Cleveland has shown that when Termites are subjected to 3.5 atmospheres oxygen pressure their intestinal protozoan fauna is killed in from 30 to 40 minutes, while the Termites themselves survive for 45 hours or longer.

Also it was found that the intestinal protozoa of the cockroaches are killed by oxygenation at 3.5 atmospheres for 3½ hours. In these forms the flagellate protozoans survive for only 40 minutes, while the ciliates live for 3½ hours. The cockroaches survive for 90 hours. Earthworms also lose their ciliated parasites when they are oxygenated. Frogs contain many protozoa; oxygenation kills Hexamitus in 5 hours, Polymastix in 7 hours, Trichomonas in 12 hours, Opalina in 18 hours, Nyctotherus in 28 hours, and the frogs themselves in 65 hours.

Comparison of Respiration in Various Groups of Animals. In order to make a comparison of the amount of oxygen taken in and the amount

TABLE 35.—TABLE SHOWING OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION IN A SERIES OF INVERTEBRATES

Species	Weight, grams	Oxygen, per kilo-hour, grams	Carbon dioxide per kilo-hour, grams	$\frac{CO_2}{O_2}$	T
Protozoa:					
Collozoum	.....	.....	0.1113	1.06	16
Cœlenterates:					
Carmania	.....	.....	0.0087	1.10	(?)16
Cestus veneris	.....	.....	0.0037	0.79	16
Echinoderms:					
Asterocanthion	.....	0.0457			
Vermes:					
Lumbricus	112	0.1013	0.108	0.77	(?)
Hirudo	2.2	0.0328	0.0312	0.69	13.5
		0.0567	0.0701	0.90	13
Mollusca:					
Octopus vulgarius	2310	0.0630	0.0745	0.86	15.5
Tethys lep	.....	.....	0.1115	0.95	16
Mytilus	.....	0.0174	0.0165	0.84	16
Ostrea edulis	.....	0.0191			
Cardium	.....	0.0210			
Crustacea:					
Astacus fluviatilis	31	0.0543	0.0642	0.86	12.5
Gammarus pulmo	.....	0.1885	0.1836	0.72	12.5
Palæmon	.....	0.179	0.201	0.83	19
Cancer	470	0.153	0.174	0.84	16
Homarus	315	0.0972	0.1054	0.80	15
Palinurus	520	0.064	0.0755	0.88	15
Hexopoda:					
Antheroca	42.5	0.840	0.916	0.79	
	39	0.687	0.767	0.81	
	40	1.170	1.189	0.73	
Melolontha	40.3	1.076	1.1699	0.79	
	37	0.962	1.092	0.82	

of carbon dioxide given off it is necessary to reduce the observed values to some common unit of measure. For convenience the amount of oxygen taken up per kilo of body weight in the course of 1 hour may be employed, and similarly the output of carbon dioxide may be shown per kilogram-hour. Table 35 is an attempt to show the rates at which the respiratory exchange is found to take place in a series of forms from different animal groups:

Oxygen Consumption and Oxygen Concentration.—The question whether the tension of oxygen in the environment has any influence upon the rate of oxygen consumption by organisms has interested many observers. Thunberg found that in 96 per cent oxygen the earthworm consumes 44 per cent more oxygen than it does in the air. Limax shows a similar increased oxygen consumption in an atmosphere rich in oxygen. Henze found that the oxygen consumption of Actinia, Anemonia, and Sipunculus is very largely influenced by the concentration of oxygen dissolved in the water in which the animals lived. The same author found, however, that in the Crustacean Carcinus, the Molluscs Aplysia and Eledone, and the fishes Coris and Sargus the rate of oxygen consumption is independent of oxygen concentration.

The following quotation from Lund gives the interpretations placed upon these facts by the investigators named:

> Thunberg interpreted his results to mean that the rate of intracellular oxidations was determined by the mass of the reacting substances according to the mass law, when the oxygen concentration was below a certain value. Above this concentration of oxygen no increase in oxygen consumption was to be expected because for all practical purposes it could be considered infinite in amount and therefore had the same relation to the reaction velocity and equilibrium conditions of the oxidation reaction as the concentration of water has to the reaction velocity and equilibrium in, for example, the inversion of cane sugar.
>
> Henze interprets his results in essentially the same way as Thunberg does but suggests that in cells where the actual rate of oxygen consumption due to oxygen deficiency is below the maximum, anaerobic processes supplement the aerobic processes and as a result the cells do not die because of too low oxygen concentration. To sum up, the results so far on lower animals indicate that the relation between the concentration of oxygen and the rate of intracellular oxidations differs in different animals, in particular with respect to the oxygen concentration at which an effect on the oxidative process is noticeable. Much greater accuracy in the methods for quantitative studies on intracellular oxidation in multicellular animals is necessary before a satisfactory statement in detail of the conditions for these forms can be made.

More recently Amberson has made a study along this same line, using Birge and Juday's modification of Winkler's method for oxygen determination. He found that in the cases of Nereis and Homarus the animals consume smaller and smaller absolute amounts of oxygen as the oxygen

tension is reduced. At every instant the oxygen consumption is directly proportional to the oxygen tension in the sea water at that instant. The diminution in the oxygen consumption is not due to the accumulation and inhibitory action of carbon dioxide in the water, since identical curves are obtained when the same animals are placed in sea water with different carbon dioxide tensions. He finds also that Limulus and Callinectes are also directly dependent upon the oxygen tension of the water. The shrimp Palæmonetes, however, appears able to regulate its metabolism down to about 50 per cent oxygen saturation of the water, extracting equal amounts in equal times. The squid Loligo shows even better regulation, down to about 30 per cent. No general law can be stated for the higher invertebrates, according to Amberson, and this fact should be recognized in all studies in which measurements of oxygen consumption are being made.

Respiration a Function of Rate of Diffusion.—There is a possibility that the power to regulate the rate of oxygen consumption is inherent in some cells. The eggs of the sea urchin maintain a rate of oxygen consumption which is, within limits, independent of the concentration of oxygen in the sea water. It may be that the failure of the simpler Metazoa to do so is due to the fact that the deeper-lying layers of cells depend upon simple diffusion for their supply of oxygen. While the free egg cells and the cells of organisms which possess well-developed circulatory systems get sufficient oxygen for their maximum requirements even from solutions containing oxygen at low tensions, the inner or deeper-lying cells of Actiniæ get their oxygen supply only from water of a much lower minimum concentration. A lowered concentration lowers the diffusion rate, so that the deeper-lying cells come to live partially, at least, under anaerobic conditions. A similar dependence of sea-urchin eggs upon the concentration of oxygen may be seen when the eggs are allowed, after fertilization, to lie in a thick layer at the bottom of the culture dish instead of being kept in motion, or being spread out over a greater area. Under the conditions provided by the massing of the eggs they soon die and disintegrate. Further proof that the controlling factor in changing or modifying the oxygen consumption in the case of Actinia is diffusion is afforded by the observation that the margin between normal oxygen consumption and that in oxygen-rich water is not widened by a 10° increase in temperature, which would make little difference in the rate of diffusion but a great difference in the rate of chemical reaction.

Reference has been made in another place to the fact that certain forms very lowly in the animal scale are provided with hæmoglobin while their very near relatives are not so favored. It has been suggested that there may be a correlation between habitat and the oxygen-carrying mechanism of such a nature that those animals living under very unfavorable conditions in as far as oxygen supply is concerned are provided with

the most efficient oxygen carrier. Certain of the worms are examples of this relationship.

Respiration of Worms.—In *Planaria agilis*, Lund found that the rate of oxygen consumption is determined by the oxygen concentration of the water in which the animal is placed, when the oxygen concentration at 20°C. drops to about one-third the normal saturation value. When animals have been kept for a time in water of low oxygen concentration and are returned to air there is an accelerated respiratory metabolism, but the degree of increase is not proportional to the length of time during which the animals are subjected to abnormal conditions. The acceleration of oxygen metabolism appears greater in starved than in well-fed animals. Miss Hyman has added the information that the amount of increase is greater with the greater amount of food ingested, and reaches its maximum immediately upon the ingestion of food. In these forms digestion is almost wholly intracellular, and occurs in the endoderm cells only. The process may require as long as 5 days for completion.

Earthworms are air breathers. The general surface of the body serves as a respiratory epithelium, being provided with an intricate network of fine capillaries, and being kept constantly moist by contact with the moist earth and by secretions from certain cells of the epidermis. It might be said that the surface of the body serves the purpose of a lung. The hæmoglobin of the blood, as it passes through the capillaries of the skin, is charged with oxygen which it receives from the atmospheric air of the burrow in which the worm lives. It is well known that after a rain in the spring or summer thousands of worms are often found upon the pavements, many of them dead or dying. It has been customary to think of them as drowned worms. Such an idea is probably incorrect. Hildrick Friend has pointed out that there are four possible explanations that may be advanced to account for these appearances: The worms may have been killed by parasites, cold, rain, or poison. While scientists may not at the present time be in a position to make a final statement in regard to the matter, it may be stated with some degree of assurance that probably the death of the worms is not due to an interference with the respiratory process. Worms can be kept for quite extended periods in water, provided it be kept cool and pure. In the author's laboratory earthworms have been kept in cold water for as long as 2 weeks without serious injury. While exact quantitative measurements have not been made, it would appear that the oxygen needs of the animals may be met by the oxygen content of the water in which they happen from time to time to be immersed, and that they may, by means of the same respiratory epithelium, make use of the atmospheric air or the dissolved oxygen of water with almost equal readiness.

Gas Exchange of Anodonta.—One of the most characteristic structures of both the freshwater and marine Lamellibranchs is the gill.

In the form under discussion, the gills take the form of two large, double plate-like structures lying at either side of the body within the mantle cavity of the animal. It has been very generally supposed that these structures have a respiratory function. The size of the structure and the amount of water which is passed over the gill would very naturally lead to the supposition that the animal is one demanding a relatively large supply of oxygen. As a matter of fact, these bivalve Molluscs lead a very slow sort of life and consume a relatively small amount of oxygen. The so-called gill structure finally turns out to be a mechanism for transporting minute particles in suspension in the water to the mouth. The respiratory function of the body appears to be carried on almost exclusively by those broad, membranous folds known as the mantle. The mantle is rather richly supplied with blood vessels and it is here that the blood comes into more intimate association with the water than in any other part of the body. The oxygen consumption of Anodonta has been determined under various conditions of fasting and feeding. While the total oxygen consumption is not large, the results have not been entirely satisfactory. Marked variations in the oxygen intake of one and the same animal have been observed under such conditions that they could not be attributed to variations in temperature or oxygen content of the water or in muscular activity.

Respiratory Processes of Mya Arenaria and Other Marine Mollusca. Some of the marine Molluscs are able to make use of the calcareous reserves of the shell and of the liver to regulate their acid-base equilibrium. This can be done with such precision that no change in the pH is brought about, even when they are subjected to the most abnormal conditions. *Mya arenaria,* under anaerobic conditions, will survive for a period of time which is dependent upon the temperature, the rate of carbon dioxide production being increased with a rise in temperature, and depressed when the temperature is lowered. Glycogen disappears from the tissues during the anaerobic periods. Potassium cyanide depresses the rate of carbon dioxide production under anaerobic conditions as well as depressing the consumption of oxygen under normal conditions. This indicates that the carbon dioxide production under anaerobic conditions is due to a series of oxidations. These forms may have some store of available oxygen which may be called upon when the outside supply of oxygen is cut off.

The Crystalline Style as a Factor in Respiration.—Berkeley is of the opinion that the crystalline style is a factor in the anaerobic respiration of certain of the marine Mollusca. In *Saxidomus giganteus* and *Paphia staminea* the crystalline style disappears entirely if the animals are kept in water which has been freed of oxygen and out of contact with the atmosphere. In *Mya arenaria* the crystalline style does not disappear, but it does become appreciably diminished in size during anaerobiosis.

Upon access to the air the style regains its normal size. The style seems to contain a substance which is a complex of an oxidizing agent and an enzyme which can convey oxygen to the tissues when the supply of atmospheric oxygen fails.

Respiration among Crustaceans.—Most members of the phylum Crustacea breathe by means of gills. These are developed in different degrees according to the need of the animal for more or less rapid gas exchanges. In the Isopods, however, some remarkable transitional conditions between the more extreme gill breathing on the one hand and the tracheal breathing of the insects are found. The wood lice belong to an aquatic family. *Asellus aquaticus* may be taken as a typical example. Next to this in habitat and general structure is *Ligia oceanica*. In these forms respiration is effected by means of respiratory organs upon the abdominal appendages which have become modified into plate-like forms. The outer plate (exopod) is the stouter and covers the endopod. If kept moist, the animal is able to absorb air by these plates. *Oniscus asellus* resembles Ligia in the general structure of its respiratory organs, but has in addition a curious breathing organ at the edge of each exopod. This is hollowed out to form a very thin-walled chamber. In *Porcellio scaber* there is a similar development. Whitish patches indicating the presence of minute air tubes excavating the exopods are found on the first two pairs of abdominal appendages. These tubes exhibit none of the perfections of the tracheæ of insects, but enable the possessors to live under much dryer conditions than other members of the group. Table 36 gives an indication of the correlation of structure, function, and habitat in this single Crustacean group.

Table 36.—Table Showing Variations in Habitat and Respiratory Organs among the Isopods

Isopod	Habitat	Respiratory organs
Asellus aquaticus	Aquatic	Gills
Ligia oceanica	High-water line	Gills with stout exopods
Trichoniscus pusillus	Wet grass	Gills with stout exopods
Oniscus asellus	Damp places	Gills with special air chambers at edges of exopods
Philosira muscarum	Damp places	Gills with special air chambers at edges of exopods
Porcellio scaber	Drier places	Air tubes in two pairs of exopods
Armadillium vulgare	Dry places—often very dry	Air tubes in two pairs of exopods—with power of rolling up

Respiratory Surface in Crabs.—In view of the importance of the exposed surface for respiratory purposes a determination of the relation

existing between surface and body weight is of real interest. Damant has given us the results of a careful determination of the respiratory surface in some of the crabs. These forms were chosen because they lend themselves to accurate measurement, the respiratory surface evidently has but one function to perform, and the integument of the animal is of such a character that it can hardly take any appreciable part in the respiratory exchange.

The gill of the crab consists of pyramidal piles of heart-shaped lamellæ resembling the leaves of a book, which in the common shore crab are comparatively rigid structures standing manipulation, alcohol, etc., without change of form. Careful count of the leaves and a measurement of the surface of the lamellæ indicate that in the shore crab, *Carcinus mænas*, the gill surface averages 7.7 square centimeters per gram of body weight; in *Cancer pagurus*, 7.8 square centimeters per gram; and in Portunis, of which only one specimen was examined, 87. square centimeters per gram of body weight. It is to be noted in this connection that the determinations show that for the smaller crabs the respiratory surface is relatively larger than in the larger crabs, a specimen of *Carcinus mænas* weighing 23 grams having 8.3 square centimeters per gram and a specimen of 102 grams having a surface of 6.6 square centimeters per gram of body weight.

These measurements are certainly in line with other facts which indicate that the smaller individuals of any species have, on the whole, the more active respiratory exchanges.

Respiration in Insects.—In most of the animals above the diploblasts the blood is the chief carrier of oxygen. Other substances are simply dissolved in the serum but the oxygen is usually loosely combined with some substance in the blood plasma or in the corpuscles. Among the insects there is a different respiratory arrangement. Even a hasty examination of a group that contains aerial and aquatic forms shows that, in general, the aerial forms develop respiratory organs by invaginations of the body surface to form lungs, and that the aquatic forms develop respiratory organs by evaginations of the surface in the form of gills. So among the Arthropods the aquatic forms are provided with gills and the aerial forms, which include most of the insects, have tracheæ. Here it would also be natural to expect to find that the respiratory system is, like other systems of the body, more or less segmentally arranged.

In the development of the very finely divided tracheal system the blood of the insects has lost one of its most important functions, that of oxygen carrying. How such a condition may have been brought about is, of course, a matter of conjecture.

Insects which live in the water form two groups:

1. False aquatics, larval or adult forms which come to the surface to breathe atmospheric air.

2. True aquatics, all larval forms specialized to make use of the oxygen dissolved in the water in which they live.

It is doubtless true that larval forms are, on the whole, most subject to modification because of the dependence of the animal upon its surroundings during the growth period. These modifications may be quite different in very closely allied forms. For example, in the two diptera Culex and Chironomus the adults are almost alike, yet the younger stages are remarkably unlike in the way they have adapted themselves to an aquatic life.

The following statement indicates some of the differences in the carrying on of the respiratory process in the larval stages of the two forms mentioned:

Culex	Chironomus
Comes to the surface to breath.	Remains entirely submerged.
Breathes by means of spiracles at the end of respiratory siphon on the eight abdominal segments.	Breathes by gills which are tubular outgrowths of the last two segments.
Active.	
Lives at the surface of the pool.	Lives in the mud at the bottom.
Has colorless blood.	Blood deeply colored with hæmoglobin.
Has well-developed tracheal system.	Tracheal system absent.
Pupæ	*Pupæ*
Respiration effected by a pair of trumpets coming off from thorax and opening to the air.	Respiration effected by respiratory tufts from the same regions as in culex—true aquatic organs.
Oxygen is in gaseous condition until it reaches the cells needing it.	Oxygen remains in solution all the time.

According to Miall, in true aquatics with tracheal respiration the oxygen is taken out of solution in the gills and carried through the body as a gas.

Respiratory Exchanges of Lower Land Animals.—The French chemist Vauquelin (1763-1829) appears to have been the first to experiment with the respiratory exchange of the lower land animals. His experiments had to do with the gas exchanges of the grasshopper and some of the snails. Experiments were also carried on by Spallanzani, Sorg, Treviranus, Neuport, and others, but their methods were so crude that it is not possible to place much reliance upon their results.

Treviranus noted the fact that carbon dioxide production increased with rising temperature and with voluntary movements. He also found that the respiratory exchanges in bees, bumblebees, and butterflies were relatively large. The respiratory exchanges of the larvæ of butterflies and beetles were of a lower order, and of such forms as the worms lower still.

It is only since the work of Regnault and Riset, however, that the technic of experiments upon respiration has been perfected to such a

degree as to warrant general acceptance upon the part of physiologists. The work of Pettenkofer and Voit in the perfecting of respiratory apparatus should be mentioned as of special significance.

Among the animals living upon the land special attention has been given to the insects. The experiments of Luciani and his students have a prominent place because of the thoroughness and care exercised in devising and carrying them out. For the record of these experiments I am obliged to quote from the account given by von Furth. The silkworm, *Bombyx mori*, was chosen as the subject of experimentation, and the respiratory exchanges were followed through all the stages of the development of life of the animal.

In the eggs of *Bombyx mori* it is possible to distinguish three periods: (1) the summer period; (2) the hibernation period; and (3) the period of development or incubation, which begins with the first rise of temperature in the spring and ends with the molting of the caterpillar, the so-called silkworm. Du Claux determined the gas exchange of the eggs during the different periods and found that during the summer period the exchange is very lively. During the autumn the respiratory activity decreases and the egg passes into its winter sleep, or period of hibernation. During this period the loss of weight by the eggs is very small and the eggs seem to withstand temperature changes and draughts of air much more readily than during the other periods of their development. The respiratory activity during the period of hibernation is adapted to the amount of oxygen available. If the eggs are placed in an atmosphere of pure oxygen the carbon dioxide elimination is relatively large, but in an atmosphere of nitrogen it is minimal. At a temperature of from 8 to 10°C. 1 kilo of eggs will in the course of 24 hours give off an average of 0.18 gram of carbon dioxide. By further cooling to 0.0° the amount of carbon dioxide is reduced to 0.05 gram per kilo in 24 hours. If the eggs are kept for a long time in the absence of oxygen they become asphyxiated. The higher the temperature at which they are kept when in absence of oxygen the more quickly will the symptoms of asphyxiation appear. During the period of development of the eggs there is also an evaporation of water. In the loss of a large amount of water from the eggs they assume a condition in which ordinary respiration appears to have ceased and in which life processes are latent. Under favorable conditions they may again be revived.

During the period of development in the spring there is an increase in the amount of carbon dioxide excreted associated with the rise in the temperature of the air. There is also a greater sensitivity to dangers of all kinds, such as changes of temperature and lack of oxygen. A short time before the crawling out of the caterpillars the respiratory activity becomes so lively that it is more than 200 times that of the eggs during the winter period at 0°. In one case noted the amount of carbon dioxide

given off by 1 kilogram of eggs arose during the course of 24 hours from 0.05 to 11.7 grams. The respiratory quotient also rises and finally surpasses unity.

Luciani and Monaco have noted also very definite day and night variations in the respiratory activity of the young caterpillars. The young caterpillars are very heliotropic and move about with much more freedom in the light than in the darkness. This activity is also expressed in the gas exchange. The fact that such changes in the carbon dioxide production of the eggs are possible indicates the untenability of the theory advocated by Molschott that the oxidative processes in the body demand direct sunlight in the same way as the reduction processes of plants.

In the course of a month the caterpillars go through a series of five molts. Each molting is preceded by a period of sleep during which no nourishment is taken in, movements cease, and there is a decrease in carbon dioxide production, which will rise again at the beginning of reassumed activity. Before the last molt the production of carbon dioxide is materially speeded up. A decrease then follows this rise in the carbon dioxide output, corresponding to the period of purgation. Two days before the caterpillar begins to spin her cocoon she loses her appetite and empties her intestine. The body of the caterpillar becomes clearer and brighter —an indication of the changes preparatory to silk production.

That the pupæ of the silkworm breathe was noted by Reamur, who found that the pupæ died when dipped in oil. Luciani and Monaco found that the carbon dioxide production by the pupæ was significant, there being 0.0113 gram per day per cocoon.

During the chrysalis stage four phases were noticeable: a period of lethargy, then a period of awakening, followed by a short period of decreased respiratory activity, and, finally, a rapid rise which precedes the coming out of the butterfly.

The carbon dioxide production of the butterfly shows day and night variations like that of the caterpillar. This periodicity appears to be associated with the different demands made upon the muscles and nervous system during these periods.

According to Paul Bert, the respiratory activity of the shedding silk butterfly, in spite of its activity, does not reach the recorded height of the caterpillar, and decreases from day to day. For example, a butterfly produced in the first day after shedding 90 cubic centimeters, on the second day 76 cubic centimeters, and on the third day 59 cubic centimeters of carbon dioxide.

Respiration of Diving Beetles.—The respiratory processes of some of the diving beetles have been studied by many investigators. Among the forms studied is Dytiscus. Dytiscus is a false aquatic form, being supplied with a complete tracheal system, the spiracles of which have moved

backward. Most of the breathing is done through the abdominal spiracles. A hollow cavity exists between the elytra and the abdomen. This cavity communicates with the atmospheric air through a cleft at the edge of the abdomen. The edge of the abdomen is covered with a fatty substance which prevents water from flowing into the cavity. Stiff hairs along the margins prevent particles from entering and air from escaping from this air cavity when the animal is submerged.

The fact that the whole respiratory apparatus is covered by the elytra renders difficult a study of the respiratory process. Van der Hyde devised a method by which it was possible to secure some information as to the nature of the process. When the animal first comes to the surface after a period of submergence, there is at first no real breathing movement, although there may be, and usually is, some exchange of gases between the air contained in the air space and the outer atmosphere. Very soon there is evidence of an active expiration of air, sometimes steadily, sometimes intermittently. Almost immediately after the expiratory movement the animal dives. The air contained within the air spaces of animals which had been submerged for various lengths of time was taken and analyzed. The results indicated that the animals make use of the air contained in the air spaces for respiratory purposes, for a regularly increasing amount of carbon dioxide was found, and a regularly decreasing amount of oxygen, depending upon the length of time which had elapsed since the animal was last at the surface. The air expired after a normal period of submersion was found to contain 1.07 to 1.63 per cent of CO_2, 13.07 to 15.29 per cent of oxygen, and 85.86 to 83.08 per cent of nitrogen. This amount of carbon dioxide is high and seems to indicate that the air contained within the air sac is a mixture of exhaled air and atmospheric air. It is probable that some of the oxygen used by the animal diffuses from the water into the air space, and it is also probably true that some of the carbon dioxide exhaled is diffused into the water while the animal is immersed. While this is true it is also true that the rate at which the oxygen diffuses into the air space is much slower than the use of oxygen by the animal. He concludes that, while the animal may have to a certain extent become emancipated from its original environment, it is still more dependent upon the air than upon the gas derived from the water.

Respiration of the Grasshopper.—It is impossible in such a text as this to give a complete account of the respiratory mechanisms and operations to be found among different types of insects. A brief account of an example or two must suffice. The respiratory function has been quite thoroughly studied in some of the Orthoptera and some of the Hymenoptera.

The respiratory system of the grasshopper has been described in some detail by Vinal. It consists of spiracles opening on the surface of the

thorax and abdomen, tracheal tubes, and air sacs. The tracheal tubes become very finely divided, extending to the deepest-lying tissues of the body and penetrating even to individual cells. The air is propelled through the tube system by the alternating contraction of two sets of muscles, one set of which, the inspiratory muscles, serves to enlarge the size of the abdomen, and the other set, the expiratory muscles, to decrease the size of the abdomen. For an account of the musculature involved in these movements see Folsom's "Entomology," third edition.

The working of the respiratory system of the grasshopper has been studied by M. O. Lee. He finds that of the ten pairs of spiracles opening upon the surface of the body the two pairs of thoracic spiracles and the first two pairs of abdominal spiracles are purely inspiratory in action. Under normal conditions the posterior six pairs of spiracles are all expiratory in action. The thoracic spiracles are provided with definite external valves which serve to occlude the openings into the tracheæ. The abdominal spiracles have no external valves but do have internal valves which are as effective as those of the thoracic spiracles in occluding the air tubes.

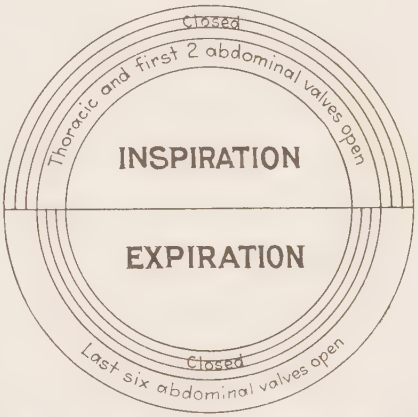


FIG. 48.—Diagram to represent the condition of the various valves guarding the spiracles of the grasshopper during the processes of inspiration and expiration.

Observations made upon the normal breathing movements of the grasshoppers show that the first and second spiracles of the thorax close tightly when the abdomen contracts during expiration, and open when the abdomen expands during inspiration. The third and fourth spiracles on the first and second abdominal segments are also exclusively inspiratory in function, though the movements of the valves are less energetic and less regular than those of the thoracic spiracles. The fifth to tenth spiracles (third to eight abdominal) act similarly and are all expiratory in character. The valve of each of these spiracles opens when the abdomen contracts and closes when it expands. Figure 48 attempts to show the relations of the positions of the valves to the inspiratory and expiratory movements. The air, therefore, normally enters the tracheal system through the four anterior pairs of spiracles, traverses the tracheal tubes, and is expelled through the six posterior pairs of spiracles. In case the four anterior pairs of spiracles are artificially blocked, the animal may inspire through the abdominal spiracles, though with some difficulty and with less than normal efficiency.

It is possible to show the effects of the breathing movements and to demonstrate the presence of carbon dioxide in the exhaled air in a very simple manner. A small hole is punched through a piece of rubber dam of such size as may be used to cover the mouth of a 20-cubic-centimeter vial. The abdomen of a grasshopper is thrust through the opening in the rubber so that the rubber fits tightly about the body of the animal between the second and third abdominal spiracles. The rubber may then be fastened over the end of the vial in one of the three positions shown in Fig. 49, *a*, *b*, *c*. The following experiments reported by Lee are suggested as being worthy of repetition.

1. The animal is placed in the tube in the *a* position, the vial being filled with an atmosphere lacking in oxygen. The behavior of the animal is normal.

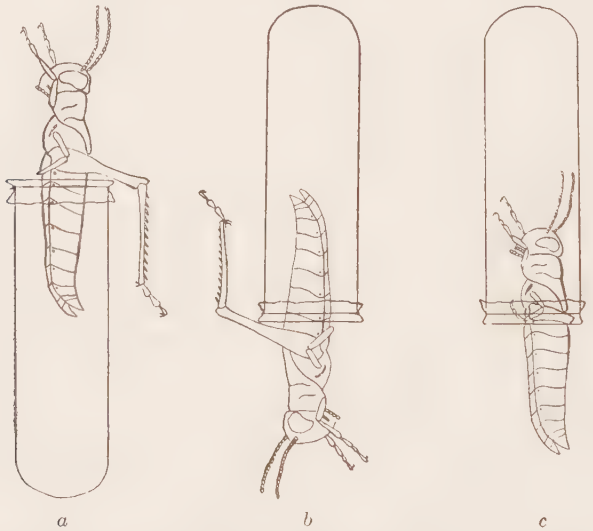


FIG. 49.—Diagrams showing grasshoppers with abdomen or head inside glass vial. A small hole is punched in rubber dam stretched over the mouth of the vial. Abdomen is thrust through this hole so that the rubber fits about the body between spiracles IV and V. *a*, expiratory spiracles inside of vial containing $Ba(OH)_2$ or air minus oxygen; *b*, expiratory spiracles inside of vial inverted and full of water; *c*, spiracles I–IV inside vial inverted and full of water, or with air minus oxygen.

2. The animal is placed in the tube in the *c* position, with the vial filled with an atmosphere lacking in oxygen. The respiratory movements are rapid and dyspnœic.

3. The animal is placed in the tube in the *a* position, the tube containing 2 cubic centimeters of $Ba(OH)_2$ solution. The liquid in the tube quickly becomes milky due to the active production of carbon dioxide by the grasshopper.

4. This experiment is similar to experiment 3, except that the animal is placed in the tube in the *c* position. No carbon dioxide is given off

to the solution in the tube. The animal breathes rapidly and gives evident signs of discomfort. This demonstrates that the anterior four spiracles are not expiratory in function.

5. This experiment is designed to show whether spiracles 5, 6, and 7 exhale an appreciable amount of carbon dioxide. The grasshopper is placed in the tube with 2 cubic centimeters of barium hydroxide solution in the *a* position, after the three posterior spiracles on each side have been cemented shut. It is found that the liquid in the tube quickly becomes clouded with barium carbonate.

6. In this experiment the tubes are filled with barium hydroxide solution, and the animals placed in the tubes in the *a* position. It is found that there is a very large exhalation of carbon dioxide from the most posterior spiracle. Plugs of barium carbonate form in all of the abdominal spiracles below spiracle 4, showing that all these spiracles serve as expiratory spiracles.

7. In this experiment the tube is filled with water and the animal placed in the tube in the *b* position. The rubber is rather loosely fastened in place so that there may be a leakage of water from the tube as gas arising from the abdominal spiracles collects in the upper part of the tube. Analyses of the gas collected in this way give an average of 4.5 per cent carbon dioxide and 15.1 per cent. oxygen.

8. This experiment is a repetition of experiment 7, but with the animal in the *c* position. In this case no gas is given off from the body, and therefore none collected. There are some signs of discomfort upon the part of the animals, but the latter are not asphyxiated. The supposition is that the abdominal spiracles serve both inspiratory and expiratory functions.

9. Animals with the spiracles 1 to 4 of the right side cemented shut are placed in the tubes in the *a* position. Bubbles of gas are seen to escape from the abdominal spiracles of both sides, thus showing that there is a definite side-to-side connection of the tracheal tubes.

Experiments are also made to determine the effects of temperature upon the respiratory process. Inasmuch as the observations made are upon the rate of the respiratory movements rather than upon the relative amounts of carbon dioxide produced at the different temperatures, the results seem to the author to be inconclusive and misleading.

Krogh found that about 20 per cent of the air contained in the hind legs of the grasshopper can be renewed in one breath. The percentage of oxygen in the air of the hind leg is very high during rest, reaching a value of 16 per cent. After muscular exertion this value is reduced to 5 per cent. The percentage of carbon dioxide found is always considerably less than the oxygen deficit, so that a large part of the carbon dioxide formed must be carried away by other means than by the tracheæ.

The Odonata (dragon flies) are among the most highly organized of the insects. Their very active existence calls for a very plentiful supply

of oxygen. This is supplied through an elaborate tracheal system. G. G. Scott has described in some detail the tracheal system of the nymph of the dragon fly Plathemis lydia. In this form the tracheal system is at first adapted to the aquatic larval life of the animal and then changes to somewhat simpler conditions in the adult. The tracheal tubes are distributed throughout the body, extending to all the deeper lying tissues and even to individual cells. It is to be regretted that Scott's observations did not extend to the actual operation of the system.

Respiration among the Bees.—Milner has studied the respiratory exchange of the honey bees. He found that a certain colony gave the results indicated in the following tabulation:

Number of individuals	Liters of gas in a ten-day period		Heat calories	Rate per gram per day
	Oxygen	Carbon dioxide		
9635	138.4	129.9	688	0.1

When this is compared with corresponding facts derived from a study of human respiration one is struck by the great activity of the bees. The average laboring man of 70 Kg. weight gives off about 4000 calories of heat per day. The heat production of the bees is equivalent to that of a man of 70 Kg. doing very severe muscular labor, and giving off heat at the rate of 7000 calories per day. Such heat production by the lumbermen of the North Woods is known and has been measured. It is possible to concede that during the most active period of the day the heat production may be double that of the average of the rest of the day, possibly as high as 600 calories per hour. This is considered to be associated only with exhausting work, almost at the limit of human endurance. This output per unit of weight is somewhat greater than that of the colony of bees as a whole, but it must be remembered that not all of the bees are engaged in excessive heat production at the same time, the rest being crowded together in the shell of the cluster or in empty cells of the honey comb, or standing quietly. The amount of work that is done by the bees that are really active is comparable with that done by man under unusual conditions and is, therefore, relatively enormous; and this is maintained not only for short periods but through the whole day and the whole winter. The figures obtained in this experiment represent about as low an expenditure of energy as is ever found in a colony of bees, except for short intervals.

Oxygen Tension in the Tissues of Certain Invertebrates.—Alde found in all of the animals which he studied a positive oxygen tension of about 1 per cent in the blood and tissues. In the cœlomic cavity of the earthworm Lumbricus the tension is low. In all the atrcheates the tension is very high. In the greater number of forms between these

extremes the individual variations are insignificant. Animals placed in atmospheres rich in oxygen tend to exhibit an increased oxygen tension in the tissues. In Lumbricus, however, there does not appear to be a definite relation between the increased oxygen tension of the air and the tension in the blood and tissues. In the tracheates, on the other hand, there is a very distinct relation, the increase in the blood and tissues running parallel to that in the atmosphere.

Respiratory Exchange of Aquatic Animals.—A considerable number of experiments have been performed by Linstedt upon aquatic animals, especially fishes, with an improved Zuntz respiration apparatus.

It was found that tench (fish very tenacious of life) were not able to live even for a short time in water which was practically free from oxygen (0.077 cubic centimeter per liter). They are, therefore, incapable of true anoxybiosis such as has been found in the case of the frog. Tench, however, did survive in water containing 0.3 cubic centimeter per liter of oxygen, while perch perished in water containing 0.5 cubic centimeter of oxygen per liter.

The oxygen requirement was also determined in relation to its dependence upon temperature, and also in connection with carbon dioxide production. Inspiration and expiration were in the case of fasting tench 6.05 cubic centimeters of oxygen and 4.77 cubic centimeters of carbon dioxide respectively per kilogram per hour at 0°C. The corresponding values at 25.1°C. were 100.44 cubic centimeters of oxygen and 88.54 of carbon dioxide. From these and intermediate data it was determined that the intensity of life processes increases with rising temperature and obeys the Van't Hoff law, which holds in the case of most chemical reactions; that is, the oxygen and energy consumption double for each 10° rise in temperature.

From experiments upon 1-, 2-, and 3-year-old tench and upon two and three summer pike it was determined that the intensity of the respiratory process in both of these fishes is directly proportional to body surface. The Bergmann and Rubner surface law (according to which equivalent amounts of energy are consumed for equal surfaces), which was originally sustained only in the case of the warm-blooded animals, was further extended in its application to tench and pike. The 1-, 2-, and 3-year-old tench consumed 103, 69, and 50 cubic centimeters respectively of oxygen per kilogram-hour, while the consumption per unit of surface (centimeter squared) fell within the narrow limits of 2.2 to 2.9 cubic centimeters for all three groups. Oxygen consumption in the case of the pike was 4.1 to 4.9 cubic centimeters per unit surface and 98 to 75 per kilograms per hour. The results show that the consumption of energy is not fully explained by heat relations. Inclusion of the rainbow trout and perch in these experiments shows the enormous difference in oxygen consumption which may appear between different

fish even though temperature and surface relations may be identical. Under similar conditions the metabolism of the trout is five times that of the tench. The difference is not due to the difference between predatory and non-predatory fish, for the perch and pike exhibit no appreciably higher rate of metabolism than the tench and are even exceeded in this respect by the carp. Abnormally high or low respiratory quotients do not of necessity correspond to actual metabolic changes. Abnormally low values result from the retention of carbon dioxide in the organism, and this condition may persist for hours after pronounced cooling. In the same way elimination of carbon dioxide may be brought about as the result of a continued rise of temperature of the organism which diminishes the capacity of the fluids and tissues of the body for carbon dioxide absorption.

Oxygen Requirements of Fish.—The work of Gardner and Leetham gives an indication of the requirements of the brown trout, a relatively active fish, for oxygen. They found that a very distinct relation exists between the amount of oxygen needed and the temperature. The following table is a summary of their observations upon this point:

TABLE 37

Weight of fish, grams	Temperature of water, degrees Centigrade	Asphyxiation point, cubic centimeters of oxygen at 760 millimeters pressure	Partial pressure, per cent of 1 atmosphere	Normal cubic centimeters of oxygen at 760 millimeters pressure, in water per liter
90	6.4	0.79	1.89	8.6
90	9.5–10	0.81	2.19	8.0
80	18.0	1.49	4.62	6.61
80	17.0	1.37	4.17	6.75
90	24.0	1.97	6.83	5.89
88	25.0	2.4	8.49	5.78

It has been further shown that, in sharks living in sea water, the oxygen tension of the blood is less than that of the water. This is partly due to the consumption of oxygen in the blood itself. The oxygen tension of fresh blood shaken with air decreases very rapidly and after 3 hours the blood is almost completely venous.

The same authors have found in the brown trout a very distinct correlation between temperature and the amount of oxygen consumed.

It will be noted that the amount of oxygen consumed approximately doubles for a 10° rise in temperature, up to 16 or 17°. Above that temperature the increase is not so great. The authors do not explain this fact. It would appear to be possible that there is at higher temperatures

TABLE 38

	Temperature, degrees Centigrade	Average oxygen consumption in cubic centimeters per kilo per hour
Seven experiments	4.1– 7.4	102.5
Three experiments	16.1–16.8	206.5
Four experiments	20.1–22.0	204.2

a destruction of some substance involved in the oxidations taking place in the body.

Gardner and Leetham also made a determination of the amount of water which it would be necessary for a trout to pass over its gills in order to provide the necessary oxygen for carrying on body processes.

TABLE 39

	7–8°, cubic centimeters of water per hour	21°, cubic centimeters of water per hour	25°, cubic centimeters of water per hour
4-inch trout	242–248	743	977
8-inch trout	51207	(16) 2676	

These figures probably represent the minimum requirements.

The fish Leuciscus survives a diminution of oxygen pressure to somewhat less than 2 per cent of an atmosphere; a reduction to 1.5 per cent leads to a condition of asphyxia. There is, therefore, no anaerobiosis, thus differentiating this fish from some of the amphibia in which there has been found the possibility of existence for a time in the absence of free oxygen.

Fish are susceptible to an accumulation of carbon dioxide, a pressure of 8 to 9 per cent being injurious to Perca, 11 to 13 per cent to Leuciscus, and 30 to 38 per cent to Carrassius. On diminishing the oxygen supplied, increasing the carbon dioxide pressure, or raising the temperature, the respiratory movements are increased. Air which is taken at the surface into the mouth of the fish does not come into contact with the gills, but serves partially to saturate the water in the mouth before passing over the gills. Under normal conditions more than 68 per cent of the oxygen breathed (in 3000 to 4200 cubic centimeters of water per hour for a 150-gram Leuciscus) may be absorbed.

According to Powers, fishes are able to survive at rather low oxygen tensions without ill effects. The species of fishes which were tested had a rather narrow optimum pH range for absorbing oxygen at low oxygen tension. The limiting factor in this case seems to be the carbon dioxide

tension of the water. The pH of the sea water with a given alkali reserve is dependent upon the carbon dioxide tension. The optimum carbon dioxide tension of the sea water for a species of fish would perhaps depend upon the alkaline reserve and the optimum pH of its blood.

Effects of Carbon Dioxide.—Carbon dioxide is a substance which must be excreted from the animal body if life processes are to continue. It is therefore not at all strange that the presence of large quantities of carbon dioxide in the air breathed by air-breathing animals, or in an uncombined form in the water which forms the natural environment of the aquatic animals, should prove very disturbing to the proper balance of metabolic activities.

For goldfish it has been ascertained, for example, that the presence of carbon dioxide in the water causes increased depth of respiratory movements, more difficult breathing, and in tensions greater than 1.7 per cent of an atmosphere produces temporary inhibition of respiration comparable with the closure of the glottis in mammals.

It has also been known for a long time that a deficiency in the amount of oxygen contained in the water will lead to what is known as surface breathing, *i.e.*, the fish comes to the surface of the water and gulps air into the mouth. This is apparently not for the purpose of bringing air into direct contact with the gill membranes, but to increase the concentration of oxygen dissolved in the water which passes over the gills. Such surface breathing can also be induced by an increase in the tension of the carbon dioxide in the water to from 3.2 to 4 per cent of an atmosphere.

When a fish is left for some time in water which contains an excess of carbon dioxide a characteristic series of symptoms of narcosis are produced. There is a cessation of voluntary movements, except when stimulated; this is followed by the loss of the head-to-stream reflex, and later a loss of equilibration. If this poisonous effect is not allowed to go too far, the fish will recover when placed in water containing oxygen and free from carbon dioxide.

Dissociation of Blood of Fishes.—Krogh and Leitch have made a study of the dissociation curves of the bloods of fishes between 10 and 20° and with and without the presence of oxygen. The tension at which the blood is half saturated with oxygen is defined as the tension of unloading, t_u, of the blood. In freshwater fishes, such as the carp, eel, and pike, which are exposed to low oxygen pressure, the t_u in the absence of oxygen is comparatively low (2 to 3 millimeters at 15°) though much higher than in pure hæmoglobin solutions, but it becomes gradually increased by carbon dioxide tensions of 1 per cent or less (7.5 millimeters). In marine fishes and in such freshwater fishes as are never normally exposed to low oxygen tensions, the t_u of the carbon dioxide per cubic centimeter of blood at 15° is nearly as high as that in mammals at 37°, namely, 18 millimeters in the

cod, and 10 to 11 millimeters in the plaice and trout, and in these also the presence of a small amount of carbon dioxide diminishes very considerably the affinity of the blood for oxygen. The dissociation curve in both cases appears to be adapted to the conditions under which the animals live.

The general significance of the hæmoglobin being present in semipermeable corpuscles and not simply dissolved in the plasma is ascribed to the fact that this arrangement makes possible the adaptation of the hæmoglobin to extremely different respiratory conditions without interfering with the general composition of the blood.

Functions of the Swim Bladder in Fishes.—Numerous investigators have studied the swim bladder of fishes with reference to the part which it may play in the welfare of the animal. This structure is a hollow sac lying in the fish dorsal to the cœlom, between the alimentary canal, from which it is derived, and the vertebral column. In some of the fishes there is in the adult a persistent connection between the sac and the alimentary canal; in others this connection has been lost after the formation of the sac as a diverticulum from the gut. On a portion of the surface of the sac there is a glandular area which is very vascular. This is variously known as the *rete mirabile*, or gas gland, or red gland. In view of the rather close resemblance between the origin of the swim bladder and of the lung it has been very natural that it should be considered by embryologists as fundamentally the same sort of structure. If this is true, it would then seem true that the lung of the air-breathing animals is an adaptation of the original swim bladder to extraaquatic living conditions. It was undoubtedly this similarity of origin that first led to the suggestion that the swim bladder might have some respiratory function.

Up to the present time, four different functions have been ascribed to this organ: (1) it has been found to serve as a sound-producing organ; (2) it serves as an accessory organ of audition; (3) it serves as a respiratory organ; (4) it serves as an hydrostatic organ. Of these, the hydrostatic function is for most fishes the most important. For many fishes the respiratory function is of great importance, or may come so to be in case the fish is thrown into unfavorable conditions.

F. G. Hall has studied the respiratory function of a number of the freshwater fishes, including perch, pickerel, bluefish, sunfish, large-mouth black bass, small-mouth black bass, sucker, and carp. He made chemical analyses of the gases contained within the swim bladders of these fishes under varying conditions, such as fishes taken from the normal aquatic environment, fishes kept in the laboratory tanks, fishes in water of low oxygen content, fishes in water of increased carbon dioxide content. The normal gaseous content of the swim bladder in the forms studied is approximately that of the atmosphere when the fishes are swimming

near the surface. Under reduced air pressure, *i.e.*, in water containing little or no oxygen, there is a reduction in the amount of oxygen to be found in the swim bladder, thus indicating that the structure really has a respiratory function. The effect of an increase in the oxygen tension of the water is to increase the amount of oxygen in the swim bladder. If the amount of carbon dioxide in the water is increased there is an increase in the amount of gas in the swim bladder, so that the fish is automatically forced toward the surface of the water. This fact points to a close relation existing between respiration and the hydrostatic function.

The question as to how the gases get into the swim bladder must be answered. In such fishes as retain a permanently open duct between the swim bladder and the pharynx, it would be possible for air to pass directly from the mouth into the air sac. To render such air in the air sac available for use in the organism for purposes of respiration would require a vascular organ similar to that which is found in the rete mirabile and which may act in both directions as well as in the one. It is certain that in the forms in which there is no open duct leading into the swim bladder there is a diffusion of gas in either direction as the conditions under which the animal is placed may from time to time determine. Dalton's law of partial pressures appears to hold here as in other cases of gas diffusion. The law states that in a mixture of gases each gas exerts the same pressure as it would exert if it alone were present in the volume occupied by the mixture. Most physiologists believe that the laws of physical diffusion explain the interchanges of gas during the processes of respiration. After making an investigation of the partial pressures of the gases of the blood and of the swim bladder, Hall comes to the conclusion that there is in some of the fishes studied (carp, bass) a physical diffusion to and from the swim bladder. In other cases there appeared to be an active secretion of gas by the swim-bladder gland. This secretion is apparently controlled through the presence in the blood of some substance which modifies its hydrogen-ion concentration. An increase in the hydrogen-ion concentration results in an increased flow of blood through the gland because of the dilatation of the capillaries of the gland, and an increase in the oxygen tension due to the local dissociation of oxygen from oxyhæmoglobin.

Respiration in Hibernating Animals.—The effects of lack of oxygen and of excess of carbon dioxide upon the respiratory rhythm of hibernating bears have been studied by Dysek.

Lack of oxygen brought about through the inhalation of hydrogen brings first of all a change in the respiratory rhythm. This has been questionably attributed to the reflex action of the hydrogen and is followed in from 10 to 30 minutes by a surprising increase in the frequency of the movements, which may be maintained for as long as an

hour. Further continuation of the experiment results in an awakening of the animal, but after access of air and an ensuing series of rapid respiratory movements the condition of respiration characteristic of deep sleep is again attained. This rapid return to the normal respiration of sleep makes it probable that the increased frequency of respiration is not due so much to the awakening as to the simple dyspnœic stimulus of the respiratory center. The presence of as much as 1.5 per cent carbon dioxide in the respired air causes an increase in the frequency of respiration (as much as twice the normal rate) which persists for more than an hour and gradually disappears with access of normal air. More pronounced acceleration (of reflex origin) may be produced by air containing 2 per cent or more of carbon dioxide; air containing from 3 to 4 per cent of carbon dioxide brings about distinct dyspnœa, which persists for a time, even with the access of normal air and is then followed by remarkable apnœic conditions. The respiratory center of hibernating animals, therefore, reacts to both lack of oxygen and to excess of carbon dioxide with distinct dyspnœic conditions.

Respiratory Exchange of the Lower Animals at Different Temperatures.—Vernon has made a careful study of the respiratory exchanges of a number of different animals at different temperatures, the results of which are summarized in Table 40. The figures given are of decimilligrams of oxygen absorbed per kilogram of body weight, and the animals are arranged in the order of their relative respiratory activity.

TABLE 40

Name of animal	10°	12°	14°	16°	18°	20°	22°	24°	Q_{10}
Salpa tilesii	12	16.5	21.5	27.5	34	40	47	55	2.96
Cestus veneris	17	23	30	37.5	45	53.5	63	74	2.79
Beroe ovata	29	42	56	72	88.5	106	126	147	3.18
Carmarina hastata	57	67	77	87	97	107	117	127	1.77
Rhizostoma pulmo	54	69	85	103	124	147	172	199	2.54
Pterotrachea cor	71	82	95	112	133	159	191	227	2.29
Salpa pinnata	53	72	93	116	142	169	199	231	2.86
Tethys laporina	131	140	151	165	183	207	236	276	1.70
Amphioxus lanc	296	368	439	511	583	655	727	798	2.03
Octopus vulgaris	915	1000	1115	1240	1410	1630	1936	2315	1.94
Heliases chromis	1095	1160	1230	1330	1465	1615	1795	2035	1.56
Serranus scriba	1075	1235	1430	1660	1900	2160	2440	2770	1.97
Average temperature coefficient for the above—2.30									
Rana temporaria	1040	1120	1180	1230	1310	1500	1780	2090	1.67

An examination of the figures presented in this table will show that changes of temperature exert a very marked influence on the rate at which

oxygen is absorbed. It also appears that in almost all of the animals examined the temperature coefficient has a value which indicates that the process examined is chemical in character, and in accord with the Van't Hoff law for the increase in reaction velocity with increase of temperature. Vernon's statement that the more highly the tissues of an animal are structurally differentiated the less the respiratory activity is affected by changes of temperature seems hardly warranted.

Rate of Oxidation in Echinoderm Eggs.—Warburg studied the rate of oxygen consumption in the unfertilized and fertilized eggs of the sea urchin Arbacia and found that upon fertilization the rate of oxidation was increased some 600 or 700 per cent. He makes the statement that this increase is not due chiefly to nuclear division, for there is no proportional increase in the thirty-two-cell stage over the eight-cell stage. Also, if cell division is inhibited by placing the eggs in hypertonic sea water the amount of oxygen consumed is not greatly altered.

An egg cell respires much more rapidly than a sperm cell, probably from 400 to 600 times as vigorously. Whether the hydrogen-ion concentration of the water is a controlling factor for the egg cell has not been determined, but Cohn has shown that for the spermatozoön the hydrogen-ion concentration is a controlling factor. Spermatozoa show very little activity and have very little fertilizing power in an even slightly acid medium, while in a neutral medium their activity and fertilizing power are increased, and come to a maximum in a solution with a slightly alkaline reaction. (For a statement in regard to the oxidative processes of egg cells before, during, and following fertilization see the chapter on Reproduction.)

Oxygen Consumption during Development.—The rate at which oxidative processes take place in developing embryos has been studied for the eggs of Fundulus, the common minnow, by Scott and Kellicott. They found that during the early stages of development, cleavage, embryo formation, etc., the hourly consumption of oxygen is less than 0.10 cubic centimeter per 1000 eggs. At the time the circulation is established in the embryo there is a marked rise in the rate of oxygen consumption. From this time on the rate of consumption of oxygen remains at a practically constant value, though with a somewhat upward trend, until shortly before the time of hatching. About 80 cubic centimeters of oxygen are consumed by 1000 eggs up to hatching. At the time when the young fish leave the egg membranes the rate rises to about 0.7 cubic centimeter per hour per 1000 embryos, and increases steadily to 1.75 cubic centimeters per 1000 embryos per hour at 6 days after hatching. By this time the most of the food yolk carried by the embryo is consumed and the rate of oxygen consumption falls off very rapidly.

The amount of active protoplasm involved in the oxidative work is at the beginning very small. Scott and Kellicott investigated this point,

using the eggs of Fundulus during the early cleavages, and determined the presence of 0.12 cubic centimeter protoplasm and 2.65 cubic centimeters yolk material, together weighing approximately 2.9 grams per 1000 eggs. At 6 days after hatching, the 1000 larvæ weigh approximately 1.8 grams, nearly all of which consists of differentiated tissues. There is thus a loss of 1.1 grams, or about 38 per cent of the original weight of the protoplasm and yolk. In effecting this transformation about 270 cubic centimeters of oxygen are consumed, or a little more than 0.25 cubic centimeter per egg.

The Flow of Oxygen through the Respiratory Epithelium.—The rôle played by the respiratory epithelium in the matter of oxygen intake has been a matter of controversy for many years. Two main views have been held by physiologists: (1) that the oxygen passed through the membrane from regions of higher to lower concentration, just as in the diffusion of other substances through animal membranes; and (2) that the oxygen is transferred through the membrane from one side to the other by a definite secretory process involving the expenditure of energy upon the part of the cells composing the membrane.

During recent years the work of Krogh appears to show that, in the resting rabbit, diffusion alone will account for the transfer of oxygen from the alveoli of the lung to the blood. On the other hand, Haldane and those associated with him have taken the view that secretion upon the part of the cells is only brought into play when there is some special need for oxygen on the part of the organism. In order to test the correctness of this latter point, Barcroft and a number of associates have made a very carefully designed and performed series of experiments. Without going at all into a discussion of the manner in which the experiments were performed it may be said that they appear to have substantiated the view that diffusion alone accounts for the passage of oxygen through the alveolar membrane. From the figures which these investigators obtained in their experiments they were able to calculate the diffusion constant for the lungs of the subject under examination. The diffusion constant is here defined as the number of cubic centimeters of oxygen which pass from the pulmonary alveoli into the blood for each millimeter of pressure difference between the two. This constant was found to have a value of about 100.

It is only fair to say that there is a certain amount of evidence pointing in the other directions. Spallita made an investigation of the gas exchange taking place in the lung of the turtle. In doing this he analyzed the bronchopulmonary air and compared it with the gases of the blood. In some cases a portion of the lung was shut off from the external air for a time and then analyses made of the air contained within the tissue. In other experiments carbon dioxide was injected into a lung and after a time the residual air was analyzed. The results obtained were held to

indicate that the gas exchange taking place in the lung cannot be solely accounted for by the laws of diffusion. In the excretion of carbon dioxide the process is purely physical, but in the absorption of oxygen specific activities come into play, which are to a certain extent independent of the laws of diffusion.

Relation of the Hydrogen Ion to Respiration.—It has been shown that in a very large number of animals the control of the respiratory movements is through the hydrogen-ion concentration of the blood. The details of the mechanisms will not be discussed at this point. It is desired here to call attention to the fact that the hydrogen-ion concentration may have something to do with the ability of the cells to absorb and utilize oxygen and to give off carbon dioxide. As an example of this Cohn has shown that spermatozoa of the starfish and sea urchin are very active in slightly alkaline sea water and that they move slowly if at all in water which is less alkaline. These movements of the spermatozoa involve the use of oxygen, or are at least accompanied by an increased use of oxygen and an increased output of carbon dioxide. Cohn has also shown that decreased oxygen consumption means longer life for the spermatozoa. It would seem as if there is here a very direct relationship between the hydrogen-ion concentration and the use of oxygen by living cells.

The same sort of relationship has been shown for the eggs of the sea urchin by Warburg, concerning which he states that oxidations in the eggs are stimulated by a decrease in the hydrogen-ion concentration, and inhibited by an increase in the hydrogen-ion concentration.

Another example which may have some force at this point is that of regenerative processes as described by Loeb in Tubularia. In these forms, regeneration is accelerated by the presence of alkali and retarded by the presence of acid. It would seem likely that the acceleration or retardation of the regenerative processes might really be due to an increased or decreased use of oxygen by the cells.

The significance of the hydrogen-ion concentration of the blood, as far as the respiratory movements are concerned, would then seem to be for maintaining in the body the proper concentration of hydrogen ions which would allow cellular gas exchanges to take place at their optimum rate and in their optimum manner. Doubt still exists as to the mechanism involved in the control of oxygen use by the cells. It is, of course, possible that a slight variation in the hydrogen-ion concentration suffices to modify the permeability of the cell membranes for oxygen, or it may be that some other substance of the cell is affected.

CHAPTER XVIII

CATALYTIC ACTIONS OF ANIMALS

The animal body is the scene of many chemical changes. These changes are of a sort that would go on outside the animal at a very much slower rate than they do inside. Chemists have found that many chemical actions may be accelerated by causing the reaction to take place in the presence, or under the influence, of some other substance called a catalyzer. In order to lay a proper foundation for what may be said about the catalytic actions which occur in the living animal, some inquiry into the nature of catalytic actions in general must be made.

Bayliss' Mechanical Scheme of Catalysis.—Bayliss[1] has given a mechanical scheme of catalytic action which will help to make clear some of the features of such action.

If a brass weight of, say, 500 grams be placed at the top of an inclined plane of polished plate glass, it will be possible to find a slope of the plane such that the weight will slowly slide down. This represents any action taking time to complete. If, now, the bottom of the weight be oiled (oil equals catalyst) the rate of its fall will be greatly increased. We see that, in either case, the weight, if placed at the top of the plane, does not remain there, but, sooner or later, reaches the bottom. It may, however, be kept at the top by some sort of catch or trigger arrangement, in which case it will remain there indefinitely until the catch is released. The amount of energy lost by the weight in its fall, being the product of its weight and the ventrical height from which it has fallen, is in no way affected by the work required to move the obstacle preventing its fall, nor is the rate at which it falls when set free.

Although the work done by our sliding weight is in no way affected by the amount of catalyst (oil) used, the rate of fall is, within limits, directly proportional to it, and this is a property of catalysts in general.

It cannot be expected that a rough model of this kind would show all the characteristics of catalytic phenomena, but there are two instructive points shown by it in addition to those already spoken of. The first is the disappearance of the catalyst by sticking to the glass as the weight slides down. An analogous phenomenon is often met with in catalytic processes, as will be seen later. The second point is one of importance with regard to certain enzyme actions; it consists in the fact that, although the presence of the catalyst neither adds to, nor subtracts from, the total energy change in the reaction, the form of this energy may be altered. When the weight falls slowly by itself, nearly the whole of the energy appears as heat due to friction along the glass plane, so that the weight arrives at the bottom with very little kinetic energy; on the

[1] Used by permission of Longmans, Green and Company.

contrary, when oiled, nearly the whole of the energy is present in the weight at the end of its fall as kinetic energy, very little friction having been met with in its descent. We may notice, also, comparing the effects of different amounts of oil, that small amounts produce a much more marked result than the subsequent addition of further quantities. This is also characteristic of enzymes.

From what has been said, it follows that a catalyst is merely capable of changing the rate of reaction already in progress. In opposition to this it may reasonably be said that a reaction does sometimes seem to be initiated. The *friction* between the weight and the glass plane may be sufficiently great to prevent movement altogether, until oil is applied. But the use of the term *friction* implies the idea of movement and the existence of forces tending to produce it. One may suppose that the weight actually does move for an infinitesimal distance, but it is at once arrested by the resistance encountered. From this point of view the definition of a catalyst would be expressed somewhat thus: A substance which changes the rate of a reaction which is actually in progress, or which is capable of proceeding without any supply of energy from without if certain resisting influences are removed. The difference between diminution of friction by oil and the removal of a catch is that in the former case the action is continuous throughout the fall of the weight whereas in the latter case the action is only momentary, at the commencement of the fall, on the rate of which it has no further effect.

Characteristics of Catalysts.—Catalytic agents, in general, present certain other peculiarities which should receive mention:

1. They do not appear chemically combined with the end products of the reaction, and unless destroyed by some subsidiary reaction appear unchanged at the end.

2. If unlimited time be allowed, the effect of a small quantity of a catalyst will be the same as that of a larger quantity.

3. The amount of change produced in a unit of time by a catalyzer is, within limits, proportional to the amount of the catalyzer present. In certain cases it has been shown that an exceedingly small amount of the catalyst is able to bring about perceptible change.

Mode of Action of Catalysts.—There is some doubt as to the exact way in which catalyzers bring about accelerations of chemical reactions. In certain cases it appears true that intermediate compounds are formed, that is, compounds are formed between the catalyst and the components of the reacting system. Such compounds are of great instability and suffer rapid decomposition, with the separation of the products of the reaction and the reappearance of the catalyzer.

In certain other cases increased rate of change appears to be due to the fact that the molecules of the reacting substances are condensed—adsorbed—upon the molecules of the catalyzer and so are brought into exceedingly close relations with each other. In such situations of increased concentration, reactions between the molecules take place at an accelerated rate, and when the adsorbed molecules of one set have

reacted upon each other the molecules of the catalyzer are freed and are again able to adsorb others.

Balanced Actions.—There is a very large group of chemical reactions in which not all of the reacting substances present are changed, but there is found, even after a considerable lapse of time, a condition of equilibrium between the reacting substances and the end product of the reaction. At the same time in the mixture synthetic and hydrolytic processes are taking place. When these two sets of processes have continued to the point where there is a constant ratio existing between the synthetic and hydrolytic operations, or between the relative amounts of reacting substances and end product, the reaction may be called balanced. Sometimes the point of equilibrium is so located as to indicate very clearly that both the reacting substances and end product are present in definite and determinable proportions; sometimes the equilibrium point is so shifted to one side as to make it a question of doubt whether such an equilibrium really exists. The position of the equilibrium point may be taken as an indication of the relative rates at which the hydrolytic and synthetic operations take place.

It will now appear that if such a balanced reaction is accelerated by a catalyzer, both the synthetic and hydrolytic processes must be accelerated or the equilibrium point will be shifted.

Enzymes as Catalysts.—The effects of the action of some of the organic catalysts have been known for a very long time, but it is only within comparatively recent years that the significance of the relation between the living cells, the catalytic substances produced by them, and the reactions for which they seem to be responsible have come to be understood.

As far back as 1830, Dubrunfaut prepared an extract of malt which had the power to convert starch into sugar. Three years later there was precipitated from such an extract, by means of alcohol, a substance which could be dried and preserved, and which had a powerful effect upon starch. This substance was termed *diastase.*

As more and more of such substances were found they were spoken of by the generic term of *ferments* on account of the similarity of their action to that of alcoholic fermentation. In the time of Pasteur, it was held that ferments could be divided into two general classes, the organized and the unorganized, the former being such substances as the yeast plant, bacteria, etc., which were supposed to carry on their peculiar activities by virtue of the fact that they were living cells, and the latter, the unorganized ferments, were such substances as pepsin, trypsin, etc., which were known to do their work apart from the cells by which they had been produced.

Although some physiologists recognized the possibility that organized ferments might owe their properties to the presence within them of soluble ferments, the double use of the word gave rise to some confusion,

and Kühne (1878) suggested that the name *enzyme* (ἐν ζυμή) be used to designate the chemical bodies either within or without the living cells which are the active agents in bringing about the observed changes. The terms *biochemical catalyst* and *biocatalyst* have also been applied to these substances. Neither of the latter terms has been generally accepted, so the use of the term *enzyme* will be continued.

An enzyme may now be defined as a catalyzer produced by a living organism. It may be possible at some time to produce such bodies in the laboratory, although it has not yet been done. If such a body is produced, it will, of course, have a right to the same name.

Yeast Fermentation.—It has for a very long time been known that when fruit juices or solutions containing sugar are exposed to the air a change called fermentation takes place in the liquid. This change is accompanied by a turbidity of the liquid, a change in taste from sweet to sharp, usually a change in odor, and by an effervescence due to the production of a gas within the liquid. The change which takes place here is a splitting of the sugar and the formation of carbon dioxide and alcohol. The brilliant researches of Louis Pasteur have made possible the present understanding of the changes involved in fermentation. The change is brought about by the agency of very minute plant cells, the saccharomyces. These are the yeast fungi and are found at almost all times floating in the atmosphere, so that, when exposed, solutions of sugar are very likely to become contaminated by them. Under proper conditions they grow rapidly and reproduce by budding. For many years it was believed very generally by biologists that yeast fermentation, and other fermentations as well, occurred because of the activity of the living protoplasm of the cells.

Understanding of the real nature of fermentative action was greatly advanced when Buchner (1906) attacked the problem of yeast fermentation. He was the first to succeed in extracting from the yeast plants a substance, to which he gave the name *zymase*, which does the same work in the fermentation of sugar as is done by the unbroken yeast plants. The discovery of zymase and the facts concerning digestion in the protozoa and lower metazoa lead to a modification of the original classification of ferments, dividing them into two classes: the *endoenzymes*, which are intracellular (yeast, bacteria, etc.); and *ectoenzymes*, which are extracellular (pepsin, trypsin, lipase, etc.).

This classification is based upon the fact that while all, or at any rate a very large proportion, of the chemical operations carried on by a living organism are to be conceived of as being the result of enzyme action, certain of these enzymes act only within the cells by which they are produced, while others formed as zymogens (inactive proenzymes) within the cells actually do their characteristic work outside of the cells producing them. The endoenzymes are, of course, the more difficult to

obtain separate from the other substances of the living cells. The ectoenzymes are secreted by special cellular groups, termed *glands*, and frequently can be easily obtained by collecting the secretion of the gland, while in other cases it may be necessary to make extracts of the glands or even of whole organisms in order to obtain the enzymes. Water is apparently the only universal solvent for enzymes. Dilute glycerin is frequently used for extracting the active substances from gland cells, in which case the water acts as the solvent of the enzyme and the glycerin acts as a preservative.

Hydrolysis.—A very large number of enzyme actions, particularly those concerned in the processes of digestion, are known as hydrolyses, or hydrolytic actions. By this is meant that, under the action of the enzyme, there is added, to the molecule acted upon, a molecule of water, and then this combined molecule is separated into two portions which may or may not be of equal size. In some of the carbohydrates the cleavage products are equal in their molecular size; among protein molecules the hydrolytic cleavage products are undoubtedly unequal in size. In this case the one or both of the cleavage products may again be combined with a molecule of water and the process of splitting repeated. This will continue until the proteins have been split to certain simple forms, the amino acids. When this stage is reached, protein digestion is said to be complete.

Characteristics of Enzymes Like Those of Catalysts.—A study of enzymes and enzyme activities has shown that these substances have much in common with the group of substances spoken of by chemists as catalyzers:

1. They accelerate (but do not initiate) chemical reactions.

2. They do not appear chemically combined with the end products of the reaction. It sometimes occurs that a smaller or larger portion of the enzyme present at the beginning of a reaction is destroyed before the reaction is completed. This, however, does not vitiate the statement just made.

3. A small amount of an enzyme, given a sufficient time, is just as effective in bringing about chemical change as a larger amount.

4. Exceedingly small amounts of enzymes suffice to bring about measurable reactions. For example, invertase is able to hydrolyze 200,000 times its weight of sacchrose; rennet is able to clot 400,000 times its weight of caseinogen in milk, etc.

5. In general, the reactions catalyzed by enzymes are reversible reactions. It is a matter of importance, therefore, to know that those enzymes which are known to accelerate a decomposition process also accelerate the corresponding synthetic process.

The Chemical Nature of Enzymes.—Much effort has been expended in an attempt to decide as to the chemical nature of these very interesting

and important substances. Some have held that some of the enzymes have given protein reactions, and so have contended for a protein composition of such substances. Others have seemed to find evidence of a double structure of enzymes, such as a combination of a protein and a carbohydrate, or a combination of some colloid of unknown composition with an active principle, or as a combination of a polypeptide and a carbohydrate. The fact that most enzymes have a very small nitrogen content speaks against the idea that they are protein in character, either wholly or in part. It also seems evident that not all enzymes have the same composition, and it may be that they belong to a class of substances as yet unknown to chemistry, containing both nitrogen and carbohydrate in their molecules.

Since 1897 Bertrand has advocated a view of the double constitution of enzymes. Bayliss quotes his statement as follows:

> One of the constituents is capable of producing to a slight degree, on its own account, the chemical reaction associated with the particular enzyme in question, but requires its activity to be augmented by the presence of another substance, inactive in itself, before its action becomes appreciable. The former, according to the case, consists of acid, alkali, calcium or magnesium salt, etc. The latter component is a more complex substance, often similar to egg albumen and colloidal in character.

General Properties of Enzymes.—All enzymes appear to be colloidal in character, and thus exhibit the following characteristics common to colloids: very slow diffusion through parchment; the development of enormous surface through their particulate condition and the resulting exhibition of surface tension, adsorption, etc.; low osmotic pressure; hysteresis, that is, a loss of activity due to some unexplained spontaneous molecular change; electrical charges of the colloidal particles; etc.

Most of the enzymes are soluble in water or salt solutions and in glycerin. This fact is made use of in making extracts of the various enzyme-containing tissues. There are certain cases, however, in which such extracts cannot easily be made, especially of the endoenzymes, in which the enzyme may be either insoluble or may be destroyed in the process of extraction from the cells in which it is produced. In such cases the juice must be expressed from the tissues by heavy pressures.

The statement has been generally made that enzymes are *destroyed by heating* to comparatively low temperatures (60 to 80°C.), and that their working properties are impaired at much lower temperature. In some instances it has been stated that enzymes have suffered loss of power even at the freezing point of water. Whether such loss of power is due to the effect of the temperature or to some other factor appears still to be a matter of question. Bayliss expresses the view that this temperature destruction may be due to some change in the other component of

the colloidal system of which the enzyme forms a part. Some enzymes have been found, under certain conditions, to withstand the temperature of boiling water. It must, therefore, appear that this heat destruction cannot be accepted as a property applying to all enzymes alike.

Enzymes generally are *precipitated* from their solutions *by alcohol*, and this fact is made use of in the preparation of purified enzymes. They are also carried down as precipitates from solutions, wholly or in part.

For each enzyme there is an *optimum temperature* at which it does its best work. As is well known, chemical processes are, for the most part, doubled or trebled in rate by a rise in temperature of 10°C. For many of the enzymes this rule appears to hold good between the freezing point of water and 40 or 50°. In most cases the activity of the enzyme is greatly reduced above 50°, and as the temperature rises the decrease in activity becomes more and more evident.

Most enzymes are very *accurately adapted to the reaction of the medium* in which they occur, or in which they are active, in nature. For example, pepsin acts to best advantage in combination with an acid. It does not digest protein, but the chloride of the protein and the products of the digestion are also chlorides. The amount of acid needed to change the free proteins and peptones into chlorides is not enough—an excess is required. The optimum amount of acid for peptic digestion is about that of $\frac{1}{10}$ normal solution of hydrochloric acid.

Zymogens or Proferments.—Many of the enzymes are formed by the secreting cells of the animal body in an inactive form called a zymogen, which may at the proper time and under the proper conditions be transformed into an active enzyme. These zymogens frequently appear in the gland or other secreting cells as small, rounded, or irregularly shaped granules. The granules appear to originate in the cytoplasm of the secreting cell near the nucleus, and in many cases become so abundant in the cell as to engorge the protoplasm with a coarsely granular mass which may become so dense as to obscure the nucleus. It seems likely that the operation of forming the granules of the cell is a progressive one. The granules near the margin of the cell exhibit frequently different staining actions from those nearer the nucleus, and they are larger in size. When the time for the elimination of the granules from the cells approaches, the granules often swell, and undergo other changes, the nature of which is not known. Sometimes the granules are not extensively modified until after they are extruded from the bounds of the cell, when they are acted upon by other agents. It should not be thought, because of the above account, that all cellular secretions are of this inactive zymogenic form. All of the enzymes which act upon the di- and polysaccharids, with the possible exception of the glycolytic enzyme of the muscles, are thrown off from the secreting cells in the active form. The same is true of erepsin and the *nucleases*. The fact that such important

substances as the enzymes are produced in an inactive form makes it necessary for the animal to elaborate some method by which these inactive substances may be rendered active. The method employed is the production of some substance, an *activator*, by the body, which will activate the zymogens. Examples of this physiological principle are shown in tabular form in the following list, which names the inactive substance, its activator, and the active product in several instances.

Zymogen	Activator	Enzyme
Proptyalin	Orokinase	Ptyalin
Propepsin	Hydrochloric acid	Pepsin
Protrypsin	Enterokinase and calcium	Trypsin
Prorennase	Calcium salts	Rennase
Prolipase	Bile salts	Lipase
Proglycogenase	Hormone from pancreas	Glycogenase

Coenzymes.—It has been shown in several instances that the activity of an enzyme may be greatly increased by the presence of some other substance termed a coenzyme. Such actions may be reversible. Examples are: laccase, whose activity is increased by manganese in small amounts; pectase whose activity is increased by calcium salts; liver lipase, by bile salts, sodium cholate, and sodium glycocholate; and zymase by disodium phosphate.

Zymogens in Protozoa.—The tryptic enzymes of both protozoa and metazoa are formed in the cells in inactive forms known as zymogens. In the protozoa granules appear in the cytoplasm, which become closely attached to the walls of the digestive vacuoles. These granules have nothing to do with the killing of the food taken into the digestive vacuoles, since they are frequently found to enter vacuoles in which the ingested organisms are already dead.

There are two well-defined periods in the digestion of food matters in the digestive vacuoles of the protozoa. The first is characterized by an acid reaction and may be considered the period of killing. During this period the granules just mentioned maintain their original firm consistency.

During the second period, which immediately succeeds the first, there is a change to an alkaline reaction, the granules swell and finally dissolve in the liquid contained within the vacuole. It appears very probable that the digestive capacity of the vacuolar liquid during the second period is a function of the dissolved granules. That is, the granules are the carriers of the tryptic enzyme. If this is true, it would then seem likely that the granules really form a preliminary stage (zymogen) of the tryptic enzyme and the acid secretion serves the purpose of changing the inactive zymogen into the active ferment. In favor of this idea, it may be said that in Colpidium it has been noticed that, so long as the reaction of the

liquid of the vacuoles is acid to neutral red, there is no evidence of digestion, but as soon as the indicator shows an alkaline reaction, digestion begins. Also it appears true in Paramœcium that digestion of finely divided egg yolk does not take place so long as the reaction of the food vacuoles is acid, but begins at once when the reaction becomes alkaline, and continues to completion in the alkaline reaction. With neutral red as an indicator this can easily be tested.

CHAPTER XIX

THE WORK OF DIGESTION

The materials commonly used for food have very large molecules; usually they are colloidal in character. The colloidal nature of the substances or the size of the molecules renders diffusion through the cell membrane very slow or impossible. It is the purpose of the digestive process, then, to break up the large molecules or particles of the food substances into smaller molecules of sorts that can be readily diffused through the membranes of the cells of the digestive tract and so become available for the various organs of the animal body. So, proteins are broken down to amino acids, starches and sugars to maltose, and fats to fatty acid and glycerin.

The substances responsible for this work are the digestive enzymes which are secreted by the protoplasm of all unicellular animals and by special gland cells of the digestive tracts of the higher animals.

Intracellular Digestion.—In most animals the work of digestion is accomplished in the interior of certain organs which have been formed from differentiated tissues. In the interior of these organs the food is subjected to the action of the digestive juices which contain the different ferments or enzymes capable of dissolving the nutritious substances, particularly the albuminous matters, and thus rendering them capable of diffusing through the cell membranes. This type of digestion is ordinarily spoken of as extracellular digestion. It is this type of digestion which has been studied by Pavlov and his followers—a whole host of physiological investigators—who have succeeded in obtaining from various portions of the digestive tract pure digestive juices, and have observed their action in vitro.

There is in the animal kingdom another type of digestion, the intracellular digestion, which is accomplished within the limits of a single cell. Classic examples of this type are found among the protozoa and Cœlenterates, and it will be found that it also occurs in other groups as well. The higher invertebrates and all vertebrates still possess, in spite of their remarkable differentiation and specialization, certain cells which have conserved their power of intracellular digestion. These cells, called *phagocytes*, envelop bacteria or other foreign cells which they find in the blood or lymph and destroy them.

In view of the great interest which has been developed in theories of immunity which depend upon the activity of the phygocytic cells of

animal bodies, it is easy to see how important becomes a knowledge of the principles which underlie intracellular digestion. How are these processes carried on in cells which are able to ingest nutritive elements? Can these cells be analogous to cells of the digestive tube of higher animals? Do they possess ferments or enzymes analogous to those which are found operative in the digestive processes of the higher animals? What conditions influence the processes of intracellular digestion? Are such cells capable of choosing their food and of distinguishing between that which is indigestible or toxic and that which is digestible and nutritious? These questions are of immense practical and theoretical interest.

Character of Food Supply Demanded.—A comparative study of the processes of nutrition as they occur among the protozoa and metazoa leads to a determination of the food habits of the animals, the classes of food substances upon which they live, and the way in which the animals proceed to utilize the different classes of food substances. It is found that, with the exception of certain flagellate protozoa—which at times carry on a sort of plant-like existence—parasitic forms, and the Suctoria, which take only the liquid substances from the protozoans which they attack, solid food materials appear to be necessary.

It is very generally held that the three great classes of foodstuffs commonly demanded by the higher organisms—the proteins, the carbohydrates, and the fats—are essential to the well-being of animals of all the different animal groups.

It does appear true that in surveying the animal series there is a difference in the degree to which certain of the food classes are employed. Among the lower forms, proteins are taken almost exclusively, and in these forms proteolysis is the most important enzyme action. Higher up in the animal scale the carbohydrates and the fats are utilized to a greater degree, and in the forms using them there is to be found an increasing emphasis upon the enzymes having amylolytic and lipolytic powers.

Peptic Digestion.—Biedermann is authority for the statement that, among the invertebrates generally protein digestion is of the tryptic type. As has already been pointed out, there is good evidence of the existence of both tryptic and peptic enzymes in all of the chief animal groups (with the possible exception of the protozoa)—though possibly not in all species. The conditions existing in the different forms, as, for example, the acid reaction of the food vacuoles in the protozoa, clearly indicate that peptic digestion would be possible if the enzyme were available. The fact that evidence of the existence of such an enzyme has been found in some forms, while not proof, is a strong indication that such might be expected in other forms as well.

The course of digestion of proteins in the higher animal forms is very well known, and there is no reason to doubt that the same general

course is followed in the digestion of the proteins by the animals lower in the animal scale.

The proteins are exceedingly complex substances, as has been previously pointed out, and are built up of various of the amino acids combined together in various ways. Under the influence of pepsin in an acid medium, these complex substances having moleculess with molecular weights of 5000 or more are changed, first, to acid albumin, and then hydrolytically split to primary and secondary proteoses with molecular weights of 2500 to 3200, and then to peptones with molecular weights ranging from 250 to 400. Authorities differ as to the percentage amount of the protein which may be transformed into peptones while the food is under the influence of the pepsin. the estimate going as high as from 50 to 57 per cent. The work of protein digestion is incomplete, however, while the food is being acted upon by the pepsin (in the stomach of higher forms), and the products of peptic activity are handed over to the intestine for the further action of trypsin and of erepsin in those forms in which it occurs.

Digestion by Trypsin and Erepsin.—Trypsin is an enzyme which acts in an alkaline medium upon all forms of protein which have been received into the intestine, and reduces them to simpler forms. There has been a great deal of discussion as to the actual extent to which tryptic digestion may split the proteins. It has been believed that the proteoses and peptones were the end products, but it is now very generally held that the digestion proceeds to the formation of the relatively simple monoamino and diamino acids. The digestion does not take place in an explosive fashion, but gradually, more as if in the nature of an erosion, and, as the amino acids are split off from the larger part of the molecule, there is a continuous absorption of the amino acids wherever these come into contact with the absorbing surface of the intestine. This results in there being only a very small amount of amino acid in the intestine at any one time. And while the process of digestion is to be considered a gradual one, it is not to be thought a slow one. Janney has shown that the time required for an organism to effect digestion of the complex protein molecule into its constituent amino acids and to metabolize them further is but a little longer than the time required for the absorption and elimination of dextrose. Van Slyke has also shown that absorption begins as soon as the food material has passed the pylorus into the intestine.

The work of the erepsin is to be considered as supplemental to that of the trypsin. While trypsin is able to work upon molecules as complex as proteins, the erepsin is able to attack simpler substances only, the proteoses, peptones, and peptides. These it splits into amino acids. It appears probable that the end result in the case of erepsin is more uniformly amino acids than is the end result of the action of trypsin. To this extent the erepsin is the more effective ferment.

Hydrogen-ion Concentration Favorable for Digestion.—Various investigators have studied the hydrogen-ion concentrations which are

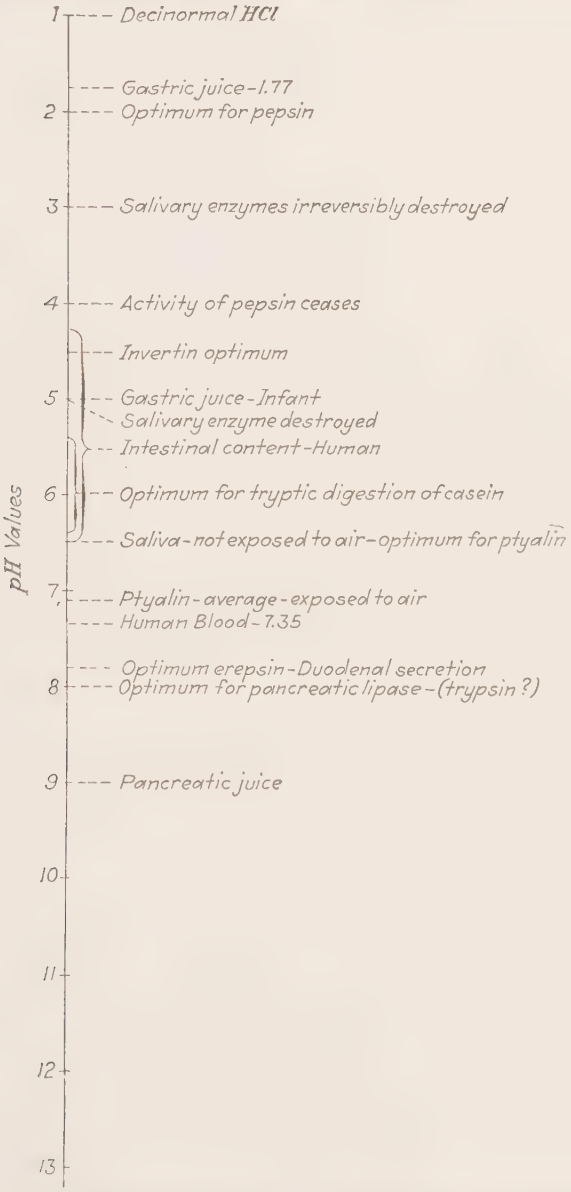


FIG. 50.—Chart indicating hydrogen-ion concentrations favorable for digestion.

favorable for the activity of the different digestive enzymes, and have attempted to correlate their findings with the hydrogen-ion concentrations found in different portions of the digestive tract. These investiga-

tors have employed different methods, some of which are justly subject to criticism, and as a result have arrived at conclusions which are far from agreement. McClendon, Bissel, Lowe, and Meyer have reviewed the literature of this subject in as far as it concerns the hydrogen-ion concentration of the intestinal content in the *Journal* of the American Medical Association for Dec. 11, 1920. Some of the more important facts in regard to the effect of the hydrogen ion are diagrammatically summarized in Fig. 50.

In addition to the three proteolytic enzymes mentioned, others occur in all the living cells of the animal organism. As the general organic matter of plants indicates an emphasis upon carbohydrate metabolism, so in the animal there appears an emphasized protein metabolism.

As will appear later in this discussion, protein digestion is carried on among the protozoa and lower metazoa, to a very large degree if not entirely, by a tryptic enzyme, that is, an enzyme active in an alkaline medium. Whether more than one type of proteolytic enzyme actually occurs in any of these lower forms may be a matter of doubt. It may be somewhat unfortunate that most of the work upon digestion has been performed by means of secretions or extracts made from the tissues of higher animals, and that there has been a tendency to assume that the enzymes of the lower forms are the same as those found in the higher.

Specificity of Enzymes.—Among the enzymes which act upon carbohydrates, considerable evidence has been found to support the idea that they are specific in their action, that is, that a given enzyme is able to effect only a certain hydrolysis or perhaps act upon a very few substances of closely similar chemical nature. Such an action would undoubtedly make itself most certainly evident in a synthetic rather than in a hydrolytic process. At any rate, students of enzyme action have determined the existence of a very large number of enzymes which are of limited, if not specific, activity.

For some reason, there is not the same degree of information as to the proteolytic enzymes. If the hypothesis is to be accepted that enzyme reactions, as applied to proteins, are reversible, as they have been found to be for other groups of organic substances, then it must be assumed that, wherever a protein is found having a definite and specific character, there must exist an enzyme capable of synthesizing that particular protein. The fact is recognized, that the protoplasm of one species of animal differs from that of another, and also that the proteins of some species have injurious effects upon the proteins of another species, while they do not have such effects upon proteins of the same species. This fact is commonly recognized by the medical profession in performing blood transfusions and in the grafting of tissues upon wounds, etc. The differences between the different proteins of nearly related species are undoubtedly slight, but they are nevertheless real, and can best be

accounted for upon the supposition that the enzymes responsible for building these different molecular structures are in themselves somewhat different. It must also be recognized that in the higher animals the proteins of one tissue are different from those of another. These differences may be very slight—perhaps less than those existing between the proteins of even closely related species. These can be accounted for also upon the supposition of slight differences in the enzymes responsible for the protein building in the different kinds of cells. It might be desirable to place the responsibility for tissue and species chemical differentiation upon some other factor, but since out of the same nutritive solution of amino acids, etc., one cell absorbs and utilizes the elements necessary to synthesize the proteins of the nerve cell and another those of the muscle cell, etc., it would seem logical to place the burden upon the character of the enzyme involved in doing this work.

Protein Synthesis and Specificity.—The action of proteolytic enzymes presents many problems of peculiar interest. The proteins are the most complex of food substances and of body components and at the same time of almost infinite variety of composition. In order that they may be utilized by the living organism they must first be subjected to the action of enzymes which are able to split the protein molecules into their peculiar and characteristic building stones, the amino acids. For this purpose there are employed, in the higher animals, a series of proteolytic enzymes, pepsin, trypsin, and erepsin. The first of these acts upon proteins and splits them to somewhat simpler products, the proteoses. Trypsin is able to act upon proteins and splits them to polypeptids and amino acids. Erepsin also acts upon proteoses and splits them to amino acids.

The Amino Acids.—For the building up of proteins, the amino acids, and not simpler substances, are required. In order to make apparent something of the possibilities of protein complexity, there is included here a table of the amino acids which enter into the composition of the proteins, and their chemical formulas.

A. Mono amino mono carboxylic acids:
   1. Glycine, $CH_2.(NH_2).COOH$
   2. Alanine, $CH_3.CH(NH_2).COOH$
   3. Valine, $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH.CH(NH_2).COOH$
   4. Leucine, $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH.CH_2.CH(NH_2).COOH$
   5. Isoleucine, $\begin{matrix}CH_3\\C_2H_5\end{matrix}\!\!>\!CH.CH(NH_2).COOH$
   6. Phenylalanine, $C_6H_5.CH_2.CH(NH_2).COOH$
   7. Tyrosine, $HO.C_6H_4.CH_2.CH(NH_2).COOH$
   8. Serine, $CH_2(OH).CH(NH_2).COOH$
   9. Cystine, $CH_2.S.CH_2(NH_2).COOH$

B. 10. Aspartic acid, $HOOC.CH_2.CH(NH_2).COOH$
   11. Glutamic acid, $HOOC.CH_2.CH_2.CH(NH_2).COOH$

C. Diaminomonocarboxylic acids:

12. Arginine, $HN = C\begin{cases} NH_2 \\ NH.CH_2.CH_2.CH(NH_2).COOH \end{cases}$

13. Lysine, $H_2N.CH_2.CH_2.CH_2.CH_2.CH(NH_2).COOH$

D. Heterocyclic compounds:

14. Histidine, $C_3H_3N_2.CH_2.CH(NH_2).COOH$
15. Proline, $CH_2.CH_2.CH_2.NH.CH.COOH$
16. Oxyproline, $CHOH.CH_2.NH.CH_2.CH.COOH$
17. Tryptophane, $C_8H_6N.CH_2.CH(NH_2).COOH$

E. Ammonia.

Fat Digestion Reversible Action of Lipase. The classic example of the reversible action of enzymes is that of the action of lipase upon a simple fat. This was first studied by Kastle and Loevenhart, and is probably now the best understood of any of the reversible actions. From a qualitative standpoint, the experiments are so simple as to make a repetition of them a very easy matter.

If to a solution of pancreas extract in a test tube a solution of ethyl butyrate is added, it is found after a short time that there has been a diminution in the amount of the ester ethyl butyrate, and that butyric acid and alcohol have been formed in the tube. When this experiment is made in a test tube, there is never a complete splitting of the ethyl butyrate. Some always remains unchanged, no matter how long the experiment be continued. If, now, a small amount of butyric acid and alcohol be added to a pancreas extract in a test tube, it is soon found that the odor of ethyl butyrate will excape from the tube. This reaction, too, is incomplete. There always remains some of the butyric acid and alcohol. These two experiments indicate that there is here what may be termed a balanced action. Neither the hydrolysis nor the synthesis can be complete so long as the substances produced by the action of the lipase remain in the tube. There comes a point of equilibrium in which all of the substances entering into the reaction appear—ethyl butyrate, butyric acid, alcohol, and water—in definite and constant proportion so long as the conditions of the experiment remain unchanged. The mode of action of the lipase appears to be by the formation of some sort of a compound between enzyme and ester on one side, and between enzyme and the butyric acid and alcohol on the other. Just what this compound may be is a matter of conjecture.

In a similar way, lipase has been found to hydrolyze the higher fats as well as the simpler esters. The synthetic production of higher esters of glycerol would therefore be expected to take place, and, as a matter of fact, has been observed in certain cases.

The Physiological Significance of Reversibility of Lipase Action.—This account of the digestion and synthesis of a simple fat by lipase is of importance because of the hint which it gives as to the manner in which fats, and possibly other substances, are handled in the body. Fats are

taken into the lumen of the intestine and there hydrolyzed. The products of this hydrolysis, fatty acid and glycerin, are diffused through the membranes of the epithelial cells, and are under the influence of the enzyme lipase, present in the protoplasm of the cells, recombined into neutral fat. The fact that lipase exists in cells where fat storage occurs has been demonstrated by Loevenhart and others, and the lipolytic action of extracts of various fat-storing tissues has come to be a matter of common laboratory experience. It must, in all fairness, be said, however, that it has proved very difficult to demonstrate any parallelism between the amount of fat secreted by the cells and the amount of lipase which can be extracted from the cells. Very small amounts of lipase would undoubtedly suffice in fat-storing cells, since fat accumulation is a very deliberate process. There is also the possibility that fat synthesis may occur in one place and storage in another. (For a more complete account see Chap. XXIII, Absorption.)

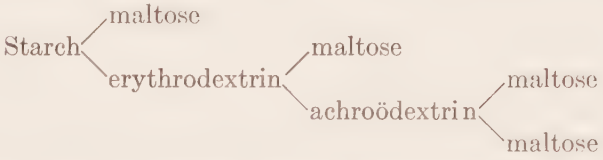
Synthetic Enzymes.—Up to the present time, no conclusive evidence of enzymes which synthesize but do not hydrolyze has been presented. On the contrary, the best evidence obtained indicates that one and the same enzyme is responsible for both processes, and that the synthetic action occurs under conditions in which the enzymes are acting in highly concentrated solutions of the products of hydrolysis.

According to this supposition, every living cell of the animal body would be provided with an enzyme or enzymes capable of synthesizing or hydrolyzing proteins, and many of them would also be provided with enzymes capable of acting upon fats and carbohydrates.

Actions of Enzymes upon Carbohydrates.—The problems associated with a study of the effects of enzymes as agents for the splitting and synthesizing of carbohydrates are very difficult, and the results of the work done have led to widely variant conclusions. Two conflicting hypotheses as to the nature of the effect of the enzymes upon carbohydrates have been presented. Van't Hoff stated that an enzyme synthesizes only the same body which under other conditions it hydrolizes. Armstrong has, as the result of his work, taken the position that an enzyme synthesizes exactly those bodies which it does not hydrolyze. It is impossible within the scope of the present work to present the evidence, much of it of a very technical nature, necessary for a discussion of the very fundamental point. In a very careful review of the whole situation, Bayliss concludes that Armstrong's view is a mistaken one and that there is no cogent evidence that enzymes produce by synthesis any bodies different from those which they hydrolyze.

Starch Digestion.—Starch is definitely a colloidal substance. During the progress of digestion it has split off from it, one after the other, molecules of maltose. The molecule of maltose is so much smaller than that of the starch as to be readily diffusible through cell membranes. The

course of events in the digestion of starch may be shown in a diagrammatic manner as follows:



Maltose acted upon by maltase becomes transformed into dextrose.

Classification of the More Common Enzymes of Animals.—The principal enzymes of animals and their functions are shown in the following table.

Enzyme	Substances Acted upon	End Product
	A. Hydrolytic Enzymes	
	I. Sucroclastic Enzymes	
Cellulase	Cellulose	Glucose
Amylase:		
a. Pancreatic—amylopsin	Starch, amylum, dextrin	Maltose
b. Salivary—ptyalin	Starch, amylum, dextrin	Maltose
Glycogenase	Glycogen	Maltose and dextrin
Inulase	Inulin	Levulose
Invertase = sucrase	Sucrose	Dextrose and levulose
Raffinase	Raffinose	Fructose and melibiose
Lactase	Lactose	Dextrose and galactose
Dextrase	Dextrose	Lactic acid
Maltase	Maltose	Dextrose
Emulsin	Glucosides	Glucose, etc.
Zymase	Glucose, Fructose, Manose, Galactose	Carbon dioxide and alcohol
Glycolytic enzymes	Sugars	Lactic acid, alcohol, carbon dioxide, water
Rhamnase	Rhamnose	
Trehalase	Trehalose	
	II. Lipoclastic Enzymes	
Lipase:		
a. Pancreatic, *b.* Gastric, *c.* Tissue	Neutral fats	Fatty acids and alcohol
	III. Proteoclastic Enzymes	
Pepsin	Proteins	Proteoses, peptones, and peptides
Trypsin	Proteins	Proteoses, peptones, peptides, and amino acids
Erepsin	Proteoses, peptones, peptides, and casein	Amino acids
Nuclease	Nucleo proteins	Purine bases
Autolytic enzymes	Tissues generally	

Enzyme	Substances Acted upon	End Product
	B. Coagulating Enzymes	
Rennase = gastric rennin	Caseinogen of milk	Casein
Rennase = pancreatic rennin	Caseinogen of milk	Casein
The above may = pepsin		
Thrombin	Fibrinogen	Fibrin
	C. Oxidases	
Catalase	Peroxides	Oxygen, water
Laccase	Polyhydric *p*-phenols, such as hydroquinone and pyrogallol	Oxidation products
Oxidases	Various tissues	Oxidation products
Peroxidases	Peroxides or hydroperoxides and carries oxygen to tissue constituents	Oxidation products
Tyrosinase	Tyrosine	Homogentisic acid
Xantho-oxidase	Xanthine and hypoxanthine	Uric acid
	D. Reductases	
Reductase	.....	Causes reduction or dioxidation
	E. Deaminases	
Guanase	Guanin	Xanthin
Adenase	Adenine	Hypozanthine
Amino acid deaminase	Amino acids	Hydroxy- or ketoacids
Urease	Urea	Carbon dioxide and ammonia
Argenase	Argenine	Urea and ornithine
	F. Carboxylase	
Carboxylase	The COOH group of aliphatic acids	Carbon dioxide

Distribution of Enzymes in the Animal Kingdom.—Table 41, gives a conspectus of the available facts as to the distribution of the various enzymes among invertebrates. It is to be noted that the investigation of the character of the enzymes found in the different animal groups is very far from complete. It is a matter of regret that knowledge concerning the range of distribution of these bodies is not more comprehensive. Much of the work from which this table was compiled is quite old, having been done before as much was known concerning the nature of various enzymes as is now known, and before the methods of research had been improved to a point where it was possible to make some of the finer distinctions in reactions as is the case at present. It is probably true that the list of enzymes found in living animals is far from complete, and that when a complete list of enzymes and the full range of these enzymes in the different groups is compiled, it will be seen that there is a

TABLE 41.—TABLE SHOWING DISTRIBUTION OF ENZYMES IN THE ANIMAL KINGDOM

	Pepsin	Trypsin	Erepain	Rennin	Lipase	Cellulase	Amylase	Maltase	Emulsin	Invertase	Inulase	Lactase	Rhamnase	Glycogenase	Fibrin ferment	Raffinase	Gentianase	Stachyase	Enterokinase
Protozoa:																			
Didymium		*																	
Æthalium	?*	*																	
Dimortha alt	?*	*																	
Vampyrella	?*	*																	
Amœba zymo	...	*	...	...															
Paramœcium	...	*	...	...	?	–	?												
Cœlenterata:																			
Sponges (sp. ?)	...	*	.	.	*	.	*												
Hydra	.	*																	
Tœdia crassa	–	*	–	*	*	.	.	–	.	–				–					
Cellaria	*	*	.	.	.	.	.												
Cliona	*	–	.	.	.	.	.	.	.	.	.	.		*					
Alcyonium	*	–	.	*	–	.	.	.	.	.	.	.		–	*				
Stomolophous meleagris	*	*	...	*	*	...	*	*	...	*t*	–	–							
Physalia arethusa	*	*	...	*	*	...	*	*	...	*t*	–	–							
Vermes:																			
Hæmophosis	–	*																	
Hirudo	–	*																	
Lumbricus	*s*	*	...	...	*	*	*												
Nereis	*s*	*	...	...	*	*	*												
Trematodes	–	–	...	.	–	–	–	–	–										
Cestodes	–	–	...	.	–	–	–	–											
Echinodermata:																			
Asterias (gland)	*?	*	...	*	*	.	*		*	*				*	?				
Asterias (stomach)	–	–	.	–	–	.	–	.	–	.				–					
Uraster	*	*	.	.	*	.	*												
Ophiocoma	*	*	...	*	*	.	*	–		*					?				
Echinus	*	–	...	*	*	*	*	*	*	*		×		×	×	×			
Thyone	.	*	.	.	.	.	–	.	.	*									
Arbacia	...	*	...	...	...	...	–	...	...	*									
Mollusca:																			
Helix pomatia	*	*	...	...	*	*	*	*	*	*	*	*	*	*	*	*	*		
Arion rufus	.	*	.	.	*	.	*	.	.	*									
Aplysia	.	.	.	.	.	.	.	.	.	.		.	*	*	*	*	*	*	
Patella	*	–	.	*	*	.	*	*	.	*		.	.	×	*	*			
Purpura	*	.	.	.	.	.	*	.	.	*									
Gasteropods (1)	.	.	.	.	.	.	.	.	*	.									
Fusus	*	.	.	.	.	.	*	.	.	*									
Mytilus	*	.	.	.	.	.	*	.	.	.									
Mya	*	.	.	.	.	.	*	.	.	.									
Lamellibranchs (1)	.	.	.	.	.	.	.	.	*	.									
Sepia	*	*	*	.	*	–	*	*	–										
Eledone	*a*	*	*	*	*	.	*	*	–										
Arthropoda:																			
Homarus	.	*	.	*	*	.	*	.	.	.	.	.	.	.	.	*	*		
Astacus	–	*	.	.	*	.	*	*	.	*	.	*	.	.	.	*	*	*	
Cancer pagurus	*s*	*	.	*	*	.	*	*	.	*	.	*	.	*	.	.	.	.	–
Maja squinado	...	*	.	*	*	.	*	.	.	*	.	.	.	.	.	.	.	.	
Portunus	*s*	*	.	*	*	.	*	.	.	*	.	.	.	.	.	.	.	.	–
Limulus	.	*	.	.	*	.	*	*	.										
Spiders	.	+	.	.	*	.	*												
Hymenoptera	.	*	.	.	.	.	*												
Blatta	.	*	.	.	*	.	*												
Pontia	.	.	.	.	.	.	.	*											
Bee	*	*	...	*	*	...	*	...	–	*	*	*							

In the above table
* enzyme present
– enzyme absent
t trace of enzyme found
? result doubtful
a autolysis
s slow
(1) genera studied not named. Probably fact stated should apply generally in group.

much more regular distribution than the table would seem to indicate. There is, of course, a rather strong likelihood that in the lower forms the enzymes are of more generalized types than in the higher forms, and

therefore able to act, less effectively, upon a somewhat wider range of foodstuffs than the enzymes of the higher animals.

In making a survey of the distribution of the various enzymes in the animal kingdom, a few important considerations are outstanding. The relative poverty of the protozoa as far as the presence of enzymes is concerned may first be mentioned. The existence of an acid reaction in the food vacuole was first interpreted to be an indication of the presence of a peptic enzyme. There does not now seem to be good ground for this belief, and it is therefore concluded that the acid reaction is to be explained upon a different hypothesis. The existence of lipoclastic enzymes among the protozoa seems to be a matter of question. It has not yet been possible to isolate a lipase, though protozoa fed upon emulsions appear to be able to utilize this as a source of food. More evidence is needed at this point. There is certainly no possibility of the existence of a cellulose-digesting enzyme in the group, though some of the protozoa appear to be able to digest cooked starches, and in some cases the digestion of the contents of plant cells without the destruction of the cell walls has been reported. Among the Cœlenterata there is a somewhat wider range of enzymic activities, though further investigation is needed here also. The digestive capacities of the worms certainly are not fully understood. Among the Echinoderms, some of the more recent work, pointing out the existence in the group of the enzymes which act upon some of the higher sugars, is certainly a matter of interest. In this group of animals, and more especially among the Molluscs and Arthropods, the occurrence of enzymes which will hydrolyze the polyoses, a thing which the higher animals are not able to do, seems to be a matter of special significance. The presence of emulsin in a large number of the marine Molluscs, with the exception of certain of the Cephalopods, is also a matter of interest, especially in view of the fact that the presence of this ferment has not yet been demonstrated in the cartilaginous or bony fishes.

Enzymes of Invertebrates.—Roaf has made an extended investigation of the existence of hydrolytic enzymes among the invertebrates and has given attention also to the optimum conditions for the working of the proteoclastic enzymes.

The enzymes were prepared by treating the minced tissue, of which it was desired to test the enzymic activity, with an equal weight of glycerin. This was allowed to stand for several weeks, and then the solid portions were removed by straining through cotton. In later experiments, attempts were made to purify these extracts by precipitating the glycerin extracts with alcohol, and after filtering off the precipitate it was extracted with water containing a little toluol, the latter to act as a preservative.

One cubic centimeter of the fresh glycerin extracts of the several glands of invertebrates was tested upon 9 cubic centimeters of 2 per cent

solutions of different carbohydrates, thus making 1.8 per cent solution. The figures in Table 42 give the percentage of glucose formed:

TABLE 42

	Starch	Glycogen	Saccharose	Maltose
Digestive gland *Cancer pagurus*	0.50	0.43	0.30	0.48
"Liver" *Patella vulgata*	0.72	0.59	0.98	0.42
Intestine *Echinus esculentus*	0.98	0.54	1.25	0.63
Disc *Ophiocoma nigra*	0.31	0.04	0.83	

TABLE 43

Gland studied	Starch	Glycogen	Saccharose	Maltose	Protein		Lipase—blue litmus milk		Rennin—oxalated blood plasma	
					HCl	Na_2CO_3	Coagulated	Turned red	Not clotted	Coagulum
Digestive gland	..	..	..	..	*N*/40	*				
Cancer pagurus	*	*	*	*	slow *	*N*/40	*	*	*	
"Liver" of *Patella vulgata*	*	*	*	*	*N*/40 *	0	*	*	0	Jelly-like
Intestine of Echinus	*	*	*	*	*N*/40 *	0 *	*	*	..	Jelly-like
Disc of *Ophiocoma nigra*	*	*	*	—	*N*/40	*N*/20	*	*	..	Slightly jelly-like
"Liver" of *Pecten opercularus.*	—	—	—	—	....	.....	Precipitated	*	..	Curdy precipitate like fibrin
Crystalline style of *Pecten opercularus.*	—	—	—	—	....	.....	.....	..	..	Jelly-like clot
"Liver" of *Purpura lapillus.*	—	—	—	—	....	.....	.....	..	..	Curdy precipitate like fibrin
"Liver" of *Trochus ziz*	—	—	—	—	....	.....	.....	..	..	Slight precipitate
"Liver" of Buccinium	—	—	—	—	....	.....	.....	..	..	Slight precipitate
Pyloric Cœca of *Asterias rubens.*	—	—	—	—	*	*	Digested	*	..	Curdy precipitate like fibrin
Mesenteric filaments	..	..	..	..	Slight	*	Digested rapidly	*	..	Coagulated
Telia crassa	..	..	..	..	*N*/40	*N*/20				
Mesenteric filaments	..	..	..	..	*	.....	.....	..	..	Curdy precipitate like fibrin
Actinia mes	..	..	..	..	*N*/40	.....	.....	*	..	
Alcyonium dig	..	..	..	..	*?	—	.....	..	..	Coagulated
Cliona celata		..	..	..	*	—				
Cellaria fistulosa	*	..	..	..	*	—				

* enzyme present, or positive result
— enzyme absent
? result doubtful
0 no test made

Table 43 gives a summary of Roaf's investigations upon the digestion of carbohydrates, proteins, fats, and coagulation. Roaf calls attention to the fact that with these enzymes the point of optimum activity lies much nearer the neutral point than is true with either mammalian pepsin or mammalian trypsin. The presence of a rennin-like enzyme has been observed by several investigators among various invertebrates, and lipase has been found widely distributed among animals and plants. The coagulation of oxylate plasma calls for further investigation, but the action is not likely to be due to a kinase. It would appear as if there were here some substance capable of acting to a certain extent like thrombin. There may be two separate enzymes pepsin and rennin, or the rennin action may be simply the first stage of the action of pepsin.

Method of Studying Digestive Activity.—Roaf has developed a method for the study of digestive activity in the lower animals which gives immediate and accurate results as to the nature of the proteoclastic ferment at work.

The method consists in staining shreds of fibrin in a solution of Congo red. The fibrin is then heated to 60 or 70° for 5 minutes. The temperature should be as low as possible so as to avoid the possibility of rendering the fibrin threads less digestible, as occurs when they are heated to the boiling point.

As Congo red turns blue with acid, it is necessary to render the solution alkaline when it is desired to compare the depth of color of an acid digestion with that resulting from a digestion in an alkaline solution. It was found easiest to do this by adding solid anhydrous sodium carbonate until the precipitate which forms at the neutral point redissolves. The depth of the red color of peptic digestion can then be directly compared to that of the tryptic digestion. Even when comparing peptic digestions with each other, it is more convenient to render them alkaline before comparing.

Experiments showed that the enzymatic action of pepsin upon a solution of Congo red fibrin follows the Schütz law, as it is nearly a function of the square root of the amount of enzyme present, while for trypsin it is roughly proportional to the amount of the enzyme present.

The method of preparation of the fibrin is as follows: The fibrin should be washed, minced, and be free from blood. To each 100 cubic centimeters of 0.5 per cent of Congo red in water add 50 grams of moist fibrin. Let stand 24 hours, and then pour the pasty mass into a large volume of water heated to the required temperature and keep there for 5 minutes. The temperature can be varied according to need, but for most purposes about 80° is best, as it fixes the dye without interfering to any great extent with the ease of digestion of the resulting Congo red fibrin. The fibrin can then be placed in a piece of cloth and washed in running water by tying the cloth on the tap and allowing the water to run slowly. After

washing, the excess of water is squeezed out and the fibrin kept in equal parts of glycerin and water. When the fibrin is to be kept for a long time a little toluol can be added to prevent the growth of molds.

A very serious objection to the use of this particular indicator is that its point of change lies far on the acid side of neutrality, its range extending from pH3 to pH5, so that an operation might appear to take place in an alkaline reaction and yet, at the same time, be quite strongly acid in character.

Roaf has also tested the digestive power of watery extracts, of the alcoholic coagula of glycerin extracts, of glandular structures of several of the animals mentioned above with the following results:

TABLE 44

	Peptic enzyme	Tryptic enzyme	Lipase	Starch splitting	Glycogen splitting	Invertase	Maltase	Rennin	Lactase
Digestive gland of *Cancer pagurus*	0	*	—	*	*	*	*	—	*
Visceral hump Patella	?	*	*	*	*	*	*	*	*
"Liver" of *Pecten opercularus*	0	*	*	*	*	*	*	*	*
Intestine of *Echinus esculentus*	0	*	*	*	*	0	*	*	*
Mesenteric filaments Tealia	0	*	0	*	0	0	*	*	0
Mesenteric filaments Actinia	0	*	0	0	0	0	0	*	0

* enzyme present
— enzyme absent
0 no test made

Certain facts taken from Roaf's work and not included in the tabular statement should be mentioned. In the Actinian Actinoloba the reaction of the tentacles, using Congo red as the indicator, was practically neutral (meaning probably a pH of between 4 and 5). The reaction of the mesenterial filaments was somewhat acid (evidently somewhat more acid than pH4).

In Echinus, the food matter was chiefly barnacles and seaweed. There were rhythmical movements of the mouth parts which were determined to have a temperature coefficient of 3.2. The food when taken into the intestine was molded into oval masses, each surrounded by a capsule. As the process of digestion proceeded, the content of the capsules become more transparent. The capsule at first was somewhat acid or neutral in reaction, there being a concentration of hydrogen ions greater than pH8. After a time it became more alkaline, less than pH8.

In *Asterias rubens*, the pyloric cæca are distinctly acid, having a pH equals 4. The rectal cæca also becomes filled with a fluid of the same acidity but in the later stages of digestion the acidity of the content diminishes.

In Porania, the pyloric cæca are acid to Congo red.

The Universal Occurrence of Enzymes.—Until within comparatively recent years there has been a division of opinion as to the manner in which the digestive processes of the lower animal forms are carried on. Even as recently as 1904, Gurwitch made the claim that the fermentative action observed in the digestion of food substances was due to the direct action of the cell plasma upon the food materials. He was unable to find any valid evidence of the existence of ferments, secreted by the protoplasm of the cells. With Buchner's discovery and separation of the enzyme zymase from yeast cells, the whole matter of the digestive activity of the protozoa and other forms maintaining intracellular digestion takes on a new significance. There appears to be no reasonable doubt that the digestive processes of the lower forms follow the same general course as the digestive actions of the higher animals. They are true chemical processes, and are accelerated, as are the similar processes of the higher animals, by the substances known as enzymes. The essential difference between the digestive processes of the lower and the higher forms consists in the fact that in the higher forms the enzymes are secreted into a medium outside of the cells and do their work out of contact with the living protoplasm, while in the lower forms the enzymes are formed, and manifest their whole activity within the living cells — but not in all cases in contact with the protoplasm. There is even among the protozoa just as truly a localization of the process of digestion as there is in the higher forms; in the first it occurs within the food vacuole in the latter within the alimentary tract.

Evolution of Digestive Enzymes.—The progress of evolution should show a gradual transition of chemical composition accompanying changes of structure. As applied to enzymes, it would be expected that the original forms of these bodies would be less specialized, acting somewhat like an acid or an alkali, and that, as the animal becomes more specialized, its enzymes would become more differentiated, so that some of them could act only upon certain definite substances, and very likely in the presence of a medium of a very definite character.

In such a scheme it would be natural to expect to find, in connection with proteoclastic enzymes, a series of enzymes ranging from those capable of acting equally well in weakly acid or weakly alkaline media through those which react better in a medium of one reaction than in another, to the more specialized ones occurring in the mammalian body, such as pepsin, trypsin, etc.

On this view, the question of the identity of pepsin and rennin might depend for its answer on what particular organism was selected as the object of study. In one animal the two might have become differentiated, while in another the two actions might be bound together. Likewise the enzymes found in any one species would depend upon the nature of the food material to which that species was accustomed, showing

lesser action on substances allied to those forming its normal food supply. The less specific the enzymes the wider, though probably less intense, their action. Some such reason must explain the presence of rennin, lactose, and possibly thrombin in organisms which normally have no need for their actions. The protein splitting which may occur in either a faintly acid or faintly alkali medium may not be due to the presence of two enzymes, but to one, and by various treatments one or the other of these actions may be diminished, thus giving rise to some of those changes in action which have been noted in experiments.

Rennase.—The nature of the enzyme commonly referred to as rennin is still problematical. It seems to be a fact that wherever proteoclastic enzymes occur some evidence of milk curdling is also found. During recent years, considerable evidence has been presented showing the great probability that the rennin and the pepsin of the higher forms are identical. It has been shown, for example, that as the proteoclastic power of pepsin is impaired the milk-curdling power is also impaired—and in the same degree. In the rennase found in the Molluscs and Arthropods a different substance is apparently being dealt with. This substance is much more resistant to heat than is the rennase of the mammals and is, at least in some cases, associated with the tryptic enzyme rather than the peptic. Among the Crustacea, the curdling effect takes place in dilute solution while the proteolytic action occurs in more concentrated solutions. Also, the proteolytic action may be changed to the coagulating by heating to 60°, or by rendering the milk faintly acid in character. This last statement would seem to render plausible the suggestion that rennase may be a separate enzyme, associated in the higher animals with pepsin and in the Molluscs and Crustacea with trypsin. Evidently much more work will have to be done before a complete understanding of the situation is possible.

CHAPTER XX

SECRETION

All protoplasmic cells have the power through their metabolic activities to produce substances which may serve some useful purpose in themselves or in other cells. Some cells may elaborate substances which may be cast off from the cells and act extracellularly, or which may be stored up within the cells and serve later as stores of energy upon which the cell may call. As examples, may be cited the power of the Amœba to build up and to secrete into its food vacuoles enzymes which may serve to digest the food materials contained therein, the ability of the salivary glands of the higher animals to secrete digestive enzymes, or the power of the highly differentiated muscle fibers and liver cells to store glycogen.

In the course of the evolutionary process certain cells have been set aside to be chiefly responsible for the production of chemical substances of a great variety. These are the gland cells.

The *secretions* discharged from the gland cells consist of water and substances built up by the activity of the cell protoplasm. The substances contained in the secretion are such as do not appear in the blood going to the gland. Definite metabolic activity is, therefore, involved in their appearance. The water of the secretion is simply the vehicle by which the secreted substances are carried away from the cells. In addition to the particular secreted substance produced by any particular gland, substances of low molecular weight may be thrown off from the gland which diffuse from the blood through the gland cells during the process of active discharge. Among such substances sodium chloride is an important one.

In the evolution of the higher animals from the lower forms there has been a great advance in the specialization of those structures known as *glands*.

It is not the purpose at this time to enter into a discussion of the microscopical structure of different types of glands, further than to make a few general suggestions. Any good textbook of histology can be consulted for further information. It is a conspicuous fact that the cellular differentiations leading to the power of the gland cell to act as a secretory organ are molecular or chemical in character rather than morphological. In a great many cases the production of a secretion is about the only evidence available that a given cell or group of cells has become highly specialized. Histologists have in their textbooks given

little attention to the differences in appearance between resting and active gland cells.

Typical Gland Structures.—Glands exist in a great variety of structural forms, ranging from a single epithelium cell to arrangements into sheets, and into simple and compound sacular and tubular structures of great complexity. The chemical work which the glands accomplish must be looked upon as their contribution to the organism, and it is of far greater significance than the structural peculiarities which the various glands exhibit. All gland structures can, in final analysis, be reduced to three elements —a secretory *epithelium cell* lying in close relations to a *blood supply*, and placed upon a *basement membrane* which serves to hold the cell in position. Every gland is formed by an infolding from an epithelium surface. The more typical secretory epithelium is found in the deeper-lying, blindly ending, terminal portions of tubules or sacs. Along the ducts there is found a less modified type of gland cells.

As examples of gland structures of different types may be mentioned: unicellular mucous glands in the lining epithelium of the intestines of all higher animals; simple tubular glands (the glands of the gastric epithelium of frog and many other animals); simple sacular glands (the skin glands of frog); compound tubular glands (kidneys); and compound sacular glands (pancreas and salivary glands).

Types of Work of Glands.—The work of the gland cells is either (*a*) selective, or (*b*) constructive.

It consists in part of removing from the circulatory fluids of the body certain definite substances. Such secretion occurs in the work of the kidney, which selects urea, for example, from the blood stream and eliminates it from the body.

Most secretion involves the elaboration by the gland cells of some chemical substance not found in the body fluids. This is chemical work, and may call for an expenditure of energy upon the part of the cells.

In many accounts of normal histology of glandular tissues little emphasis is given to the fact that a secreting cell may have a very different appearance when at rest from the appearance it has during or immediately following the process of secretion. During periods of rest the protoplasm of the gland cells becomes filled and in many cases distended with numerous granules. The nucleus of the cell is frequently observed by these granules. During secretion the granules are lost from the cell, the protoplasm becomes clearer, and the cell shrinks markedly in size.

Secretion a Sign of Irritability.—This chemical work upon the part of the gland cells is a sign of the irritability of the cell, just as truly as that the contraction of a muscle or the passage of a nerve impulse is an indication of the irritability of muscle tissue or of nerve tissue. The response of the muscle fiber or of the nerve cell is in accord with what is known as the *All or None Law*. It would certainly be interesting to

discover whether glandular secretion follows the same law. The only observation which the author recalls which may bear directly upon this point is in connection with certain pigmented structures in the integument of certain aquatic worms. When the animal is subjected to chemical stimulation the pigmented structures discharge their pigment into the surrounding water. No case has been observed in which there was a partial discharge. The pigment was either all discharged or none left the cells.

The work of the secretory cells occurs in two phases: (1) active secretion, involving a considerable flow of water; and (2) recovery, in which specific substances are produced in the cells. Those phases in the work of the secretory cells are analogous to the contraction and relaxation phases of muscle, or the passing of the nerve impulse by a nerve cell and its recovery. The real constructive work of secretion occurs during the *recovery* phase. This phase may be continued in a resting stage of shorter or longer duration. Production of the secreted material ceases after a time because of the accumulation of the products in the cell. When the amount of secreted material stored in the cell is reduced by outflow from the cell the constructive phase again begins. The phase of active secretion is that in which there is being eliminated from the cell something which may have been the result of long-continued expenditure of energy.

How Is Secretion Induced?—The stimuli which are commonly employed in the animal body to bring about the production of a glandular secretion are: (1) nervous action, and (2) chemical stimulation.

Secretion through Nerve Stimulation.—It is not possible to refer to more than a few cases in which the secretion of a gland is brought about through nervous stimulation. Many physiologists believed that the secretion of the gastric juice by the gastric glands is under nervous control. The demonstration that this is true was made by Pavlov. He showed that there are in the vagus nerve secretory fibers, the stimulation of which leads, after a long latent period, to activity on the part of the gastric glands, and the discharge of gastric juice into the lumen of the stomach. Pavlov showed that the result was essentially the same whether the glands were stimulated electrically or by the natural stimulus. He prepared animals for experimentation by dividing the œsophagus in the neck and bringing the two cut ends to the surface in separate openings. It was thus possible to give to an animal the full enjoyment of eating without having the food pass directly to the stomach. Or, it was possible to pass food into the stomach of the animal without having it pass through the mouth. It was found that if the animal is allowed to eat and swallow food—to the exterior—there is an abundant secretion of gastric juice, resulting from the stimulations of taste, odor, etc., acting reflexly. Pavlov referred to this type of secretion as *psychical*. Carlson speaks of it as the *appetite secretion*.

Another good example of secretion due to nervous stimulation is that of the salivary glands. In the case of the dog, the submaxillary gland is supplied by two nerves: (1) the chorda tympani, a branch of the seventh cranial nerve, which crosses the tympanic cavity and enters the fifth nerve; and (2) the sympathetic nerve. As the result of careful experimentation it has been found that stimulation of the chorda tympani nerve produces a copious watery secretion, and that stimulation of the sympathetic nerve produces a thick, viscid secretion. This result was interpreted by Heidenhain to mean that there are in these nerves two sorts of fibers: (1) secretory fibers, leading to the secretion of water and salts; and (2) trophic fibers, leading to the production of the specific solids appearing in the secretion. Babkin has succeeded in showing that: (1) when hydrochloric acid is placed in the mouth, the *secretory* and *vasodilator* nerves are stimulated, while the trophic nerves are not, and the secretion is thin and watery; and that (2) when meat powder is placed in the mouth there is stimulation of *secretory* and *trophic* nerves, but not of vasodilator fibers, which results in the production of a thick, viscid secretion.

Rôle of Muscle Fibers in Gland Action.—Mathews has given attention to the part played by contractile fibers in the processes of secretion. He points out that the effects of nervous stimulation of such structures as the salivary glands are not confined to the secreting cells. In the gland there are also muscle fibers, and these are brought into play through stimulation of the nerves. Evidences of this fact are seen, Mathews says, in the sudden gush of saliva; its sudden cessation, however prolonged may be the stimulation; the diminution in the amount of saliva secreted when the stimulations are rapidly repeated; the apparent paralysis of the nerve when the ducts are empty and its restoration to power if the ducts be passively redistended. These facts are more easily explainable upon the assumption that the muscle elements play an important part in the process of forcing the secretions from the gland.

To say that the contraction of muscle fibers is the cause of secretion seems to be mistaking the order of events. The work of secretion is chemical. No discharge of secretion can occur before it has been formed by the gland cells. That the muscle cells may play an important rôle in helping to squeeze secretions out of saccular or tubular glands seems beyond question. Mathews calls attention to the unicellular glands of the carp louse *Argulus foliaceous*, which, according to Nussbaum, are emptied by the contractions of muscle fibers surrounding them. Unicellular glands in the mantle of the Mollusc Aplysia are also surrounded by muscle fibers. Muscular fibers also occur in the livers of Gasteropods, land and water Isopods, Amphipods, and Decapods, and in all probability play some part in forcing the glandular secretion out of the cells.

Mathews says:

The poison glands of amphibia and reptilia and others of the salivary glands are provided with their own musculature, or are emptied by surrounding skeletal muscles. Many anal and cloacal glands, sweat, and sebaceous glands are provided with a musculature lying between the basement membrane and the cells. There is little doubt that the secretion of sebum is forced out by the action of this muscle. The same can be said for the secretion of the oil gland of birds. Probably the most interesting secretion involving muscular action, outside of the salivary glands, is found in the mammalian sweat glands. From the observations of Ranvier, Joseph, and others, certain secretions of sweat are probably due to the compression of the gland by this muscle. Probably the postmortem sweat secretions, secretions after closing the artery, or after the injection of strychnine are due to this cause. (There is, however, a second sweat mechanism associated with vasodilation.)

Many more examples of the muscular mechanism of secretion might be given, but these suffice to indicate the very wide distribution of such a mechanism. Muscular mechanisms are, possibly, more common among the invertebrates, but they play, also, a not inconsiderable part in vertebrate secretions. The vertebrate, however, with its delicately coordinated, closed vascular system, develops a second mechanism, that of osmosis, which we will now consider.

Such mechanisms may be more important among the invertebrates than among the vertebrates where there is really greater opportunity for the display of osmotic phenomena.

Mathews indicates that:

There is reason to believe that the mammalian sweat glands also have a double mechanism of secretion, a muscular and an osmotic. These glands are surrounded by a sheath of muscle fibers lying, like those of the skin glands of amphibia, between the cells and the basement membrane. From the observations of Ranvier, Joseph, and others, who have shown that upon stimulation of the sciatic this muscle contracts, there can be little doubt that a secretion may thus be formed. Probably sweat secretions ensuing coincident with vasoconstriction, upon the injection of strychnine, upon stimulation of the sciatic in the amputated limb, or after compression of the blood vessels are due to this mechanism.

On the other hand, certain secretions of sweat are too copious to be due to muscular constriction of the gland. That those secretions probably fall under the second, or osmotic, mechanism is shown by the following facts:

1. The coincidence of vasodilation and sweat secretion. Most sweat secretions are normally accompanied by vasodilation. If the cervical sympathetic of the horse be severed, strong hyperæmia and sweating occur on the side of the neck the nerve governs. This sweating ensuing after nerve division can hardly be explained, on the basis of secretory cell activity.

2. Pilocarpine, which does not cause contraction of the muscular sheath, causes a profuse secretion.

3. The vasomotor and secretory fibers in the cat follow the same paths.

4. Pilocarpine causes sweat secretions 14 days after nerve degeneration.

5. If the blood supply be cut off, on readmitting the blood after 30 minutes, a spontaneous secretion occurs. The similar secretion in the submaxillary is invariably accompanied by vasodilation.

6. Increasing the capillary blood pressure or drinking large quantities of water increases secretion.

The facts, as far as they go, are the same as those observed in the cerebral salivary secretions and pancreatic secretion. They justify us, in classing all three secretions in the same category. That these sweat secretions are of an osmotic character would thus be indicated. That other sweat secretions are due to muscle there can be little doubt.

Secretion through Chemical Stimulation.—A very beautiful example of the control of secretion through chemical stimulation is that of the control of the production of gastric juice through gastric secretin. As has already been mentioned, the first secretions of gastric juice by the gastric glands is due to stimulation through the vagus nerve. This secretion does not, however, continue throughout the whole course of gastric digestion. It usually ceases after a few minutes. The organism has found it necessary to devise a method which will automatically provide for the continuous stimulation of the gastric glands, over quite long periods, in order to provide sufficient gastric juice to complete the work of digestion. Edkins, Starling, and others have contributed to our understanding of this method of control. Many food substances contain substances known as *secretogogues*, substances which stimulate certain cells to secrete. Many of the condiments belong to the secretogogue class. Secretogogues are also produced in the course of protein digestion in the stomach. These secretogogues act upon certain epithelium cells in the pyloric region of the stomach. As a result of their stimulation these epithelium cells produce a substance which is termed gastrin or gastric secretin. The exact chemical nature of gastrin has not been determined, but it seems to be a basic substance related to histamine. This substance diffuses rapidly from the epithelium cells into the blood stream and is carried in the general circulation to all parts of the body. Some of it appears in the capillaries of the stomach wall, where it acts directly upon the cells of the gastric glands and causes them to produce their gastric secretion.

A very similar control exists for the pancreas, and probably for the liver.

Kinds of Substances Secreted.—The substances secreted by different animal glands serve a great variety of purposes.

1. Mechanical, to serve for:
   - Connection—served by substances secreted in fibrous form, connective tissue threads, byssus, etc.
   - Support—calcareous or siliceous spicules, cartilage, bone, chitin, dentine, enamel, silk, spider webs, spongin, trepang, conchiolin, pearl, collagen, chondrin, cellulose, etc.

Lubrication—mucous, synovial fluids, pericardial fluids, serous fluid, etc.
Insulation—fat, oils.
Protection—exoskeletal structures of various sorts.
Temperature regulation—perspiration.

2. Chemical, to serve as:
Catalytic agents—enzymes, of which there is a great variety.
Regulators of metabolism—hormones, vitamins.
Protection—dyes, corallin, murexide, etc., toxins, antitoxins, excretions.

Among the substances secreted by animals there should be mentioned a variety of animal poisons, and special secretions. These are to be found widely scattered among the phyla of the animal kingdom from the Cœlenterata to the Mammalia. Among them may be mentioned the active poisons found in the nematocysts of the Cœlenterata. These highly specialized cells which appear in great abundance upon the tentacles of Hydra, Hydroids, Medusæ, Siphonophores, etc., secrete a substance known as hypnotoxin.

Substances of a poisonous nature are secreted also by Echinoderms, Planarian worms, tapeworms, Filaria, Trichina, Ankylostomum, by various Molluscs (especially Mytilus), Arachnids, Myriapods, Lepidoptera, bees, wasps, Coleoptera, not to mention Amphibia, Reptilia, etc.

The purple dye secreted by Purpina, Murex, etc. and the ink secreted by Octopus, Loligo, Sepiola, Spirula, and Argonauta are special cases of great interest. The mucin secreted by snails, secretions of nidamental glands of Cephalopoda, the byssus secretion of mussels, the silk secretion of silkworms, wax secretion by the honeybees, by bumble bees, and other forms are types of secretion not found among the higher animals, but interesting adaptations of cellular activity to meet certain needs of the animals producing them.

The Period of Discharge.—Following an appropriate stimulation, the gland cells discharge their secretion to the outside, *i.e.*, to the lumen of the tube or sac in whose wall they lie. This period of discharge may involve, so far as the cells are concerned, three possibilities.

1. Products elaborated by and stored within the cells may simply be eliminated. The passing of digestive ferments into the food vacuoles in Paramœcium is an example of this type.

2. Cytolysis of a considerable portion of the cytoplasm of the gland may occur, while a small portion of cytoplasm and the nucleus remains and may regenerate the lost cytoplasm and renew the mass of secretion material which was lost. This takes place in many glands of the mucous type.

3. The whole gland cell may be destroyed, and its substance may become a part of the secretion. This occurs in sebaceous glands, and sometimes in mammary glands.

Period of Recovery.—During the period following an active discharge of secretion by gland cells there is a period of recovery and there may be a

more or less prolonged period of rest. At the close of a period of active discharge of secretion the cells are left with a very clear protoplasm free from granular contents, usually a cytoplasm reduced in amount, and sometimes a shrunken nucleus.

With respect to the appearance of their product, secreting cells may be divided into two general classes: (1) mucous cells, those which form thick secretions; and (2) serous cells, those which form thin, watery secretions.

The mucous cells begin the process of secretion with the formation of mucigen granules in the outer ends of the cells, near the free surface. These droplets become changed into clear droplets of mucous. The accumulated mass of secreted material in the outer ends of the cell is very sharply marked off from the deeper-lying protoplasm. Among mucous cells there may be an elimination of secreted materials at the same time that new granules are being formed in the protoplasm at the basal end of the cells.

In the basal ends of the serous cells are certain structures known as mitochondria, filaments lying in the cytoplasm near the nucleus. Small portions of the mitochondria are cut off and form the beginnings of the granules. As the granules pass from the ends of the mitochondrial rods to the outer ends of the cells, they increase in size, partly perhaps through the taking up of water, and partly through transformation of substance. They lose their original staining properties and are transformed into drops of secretion. Finally, the droplets of secretion flow together and are discharged from the free ends of the cells. Some of the mitochondrial material remains behind and serves as the source of further secretion.

Change of Permeability during Discharge.—The fact that the discharge of the secretion frequently occurs as the result of direct stimulation, either chemically or nervously, leads to the question as to what may be the effect of the stimulus upon the cell. There is, first of all, an alteration in the permeability of the cell membrane, and particularly of that part of the membrane over the distal end of the cell. Here the permeability is increased so that substances which heretofore have been retained within the cell are now enabled to make their escape. Only the outer wall of the cell is thus modified, since the secretion always leaves the gland cell in the direction toward the lumen of the tubule or saccule.

There is some evidence in favor of the idea that in some cases the active secretion of a cell involves the actual breaking down of the outer portion of the protoplasm of the gland cells rather than merely a change in the permeability of the surface membrane (see Fig. 51). In such a case the secretion granules are cast into the duct of the gland, there to break up into smaller molecules.

The zymogens are apparently complex molecules, of high molecular weight. At any rate, they do not diffuse readily from the cell. When

the cell is stimulated, however, there is possibly a splitting of the more complex molecules of the rather inert secreted substance so that smaller molecules are formed. This change would make diffusion from the cell more readily possible, and at the same time the osmotic pressure of the substance within the cell would be increased. The result of this increase of osmotic pressure would be to cause a diffusion of water from the blood

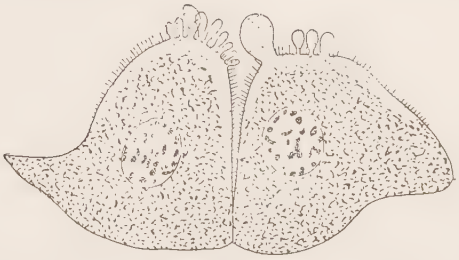


FIG. 51.—Larva of *Musca vomitoria*. Two midgut cells during the process of secretion. (*From Pütter's "Vergleichende Physiologie."*)

into the cell. As the water passes through the cell it aids in carrying the secretion through the more permeable wall. As the secretion reaches the lumen of the tube, there is in some cases a further breaking of the more complex molecules into simpler ones, with another increase of osmotic pressure. This is accompanied by a greater outflow of water from the blood stream through the cell to dilute the secretion in the lumen of the tube.

CHAPTER XXI

THE NUTRITION OF ANIMALS

The purpose of nutrition is to provide (1) the energy for running the vital machine and the material for maintaining body heat; (2) the materials needed for the renewal of worn-out protoplasm; and (3) materials for growth.

Differences between Living and Lifeless Machines.—In the first place, the materials for repair and for furnishing energy are supplied separately in the lifeless machine; in the living machine both are taken in as food. In the organic or living machine, combustions take place at a slow rate and at a relatively low temperature; in the lifeless machine, they usually take place at a much faster rate and at a higher temperature. It will be noted also that, in general, the living machine has a higher degree of efficiency than the non-living machine.

Elements Demanded in the Food Supply.—It will be recalled that in a previous chapter attention was called to the fact that protoplasm contains a very considerable number of the chemical elements, particularly those having the lower atomic weights. In that connection the fact was mentioned that more than 95 per cent of the substance of living matter is made up of four elemental substances—oxygen, carbon, hydrogen, and nitrogen. Among animals the metals calcium, potassium, magnesium, silicon, and sodium are always present, though in small amounts. Other substances are also found in varying, though always minute, amounts. It must, of course, be evident that these substances must appear in the food materials taken in, that they must be in such forms as will at the same time be harmless and capable of being worked over into absorbable forms by the processes of digestion. It is a matter of common knowledge that, in general, animal cells are not able to make use of the elements in their elemental conditions as food substances, but that they must be provided for the organism in the form of certain rather highly complex chemical compounds, proteins, carbohydrates, and the fats.

Complexity of Foodstuffs Needed.—It has been commonly believed and as commonly stated that one of the chief distinctions between animals and plants lies in the fact that the animals depend upon highly organized foodstuffs as their source of supply. Even so recent a volume as the third edition of Bayliss's "Principles of General Physiology" makes this statement:

The animal organism, even in its lowest forms, the protozoa, is satisfied with nothing less complex than glucose as a source of carbon. As regards nitrogen, the requirements appear different according to the purpose to which it is to be put, growth, maintenance, or source of energy. The experiments of Grafe (1912) appear to indicate that ammonium salts, in the presence of excess of carbohydrate, may replace wear and tear in the dog and pig, although no tissue is laid on.

The protozoa are apt to be considered as very primitive organisms, rudimentary ancestors of higher animals, because they are unicellular. But although there is no doubt that higher animals have arisen in the course of evolution from simple creatures of this kind, one must admit that the protozoa, as we have them now, are complex, highly differentiated organisms. The amœba, apparently, cannot be grown on a culture medium unless it is supplied with bacteria, although dead ones suffice.

As a general rule, we may say that animals, at all events the higher ones, require food which has been previously built up by the plant. They feed either upon vegetable matter or upon other animals.

It was believed at one time that animals, at all events the higher ones, required nitrogen in the form of more or less complex proteins, but we now have definite proof that the products of hydrolysis of proteins, amino acids, will prevent loss of nitrogen from the adult animal.

In view of the practically universal acceptance by biologists of the ideas contained in the above quotations, it is a matter of great interest to refer to the very recent work of R. A. Peters, who has shown that the protozoön Colpidium may be cultivated successfully upon a sterile culture medium of the following composition:

Sodium chloride	0.06 per cent
Potassium chloride	0.0014 per cent
Calcium chloride	0.0012 per cent
Basic sodium phosphate	0.0001 per cent
Acid potassium phosphate	0.0001 per cent
Magnesium sulphate	0.001 per cent
Sodium bicarbonate	0.002 per cent
Phenol red	Trace
Glucose	0.03 per cent
Histidine	0.01 per cent
Arginine	0.01 per cent
Leucine	0.01 per cent
Ammonium lactate	0.003 per cent
Ferric chloride	Trace
Potassium iodide	Trace
Manganous chloride	Trace

Various modifications of the culture fluid were made. The experiments seem to indicate that, when the single amino acids histidine, arginine, leucine, are supplied, there is a much more rapid growth than when

tryptophane is given; also that galactose and fructose, but not maltose, can be substituted for glucose, in the medium.

As would be expected, no growth will take place in the absence of the phosphate. Ammonium glycerophosphate (Kahlbaum) was found to serve as a complete source of nitrogen, carbon, and phosphate. Ammonium glycerate plus ammonium phosphate will serve in the same way. Nitrogen, therefore, need not be supplied in a form more elaborate than ammonia. The following table is a summary of the results of substituting various organic bodies in the place of the glucose, amino acids, and lactic acid forming the carbon source of the medium above mentioned.

Source of carbon	Growth	Source of carbon	Growth
Carbonate	Nil	Glycero phosphate	Yes
Formate	Nil	Citrate	Nil
Oxylate	Nil	Tartrate	Yes
Glycollate	Nil	Glucose and lactate	Yes
Glycerate	Yes	Leucine (synthetic)	Yes

The author states "It is quite possible that there may be means of making Colpidia live on formates, oxylates, or glycolate, but the results as they stand show that these protozoa are not dependent upon complicated chemical bodies for their growth. They show a remarkable independence." This is also the conclusion reached from some experiments performed upon the effect of varying some of the inorganic salts. The organism, for instance, seems to be widely independent of extensive variation in the ratio between calcium and potassium salts, though apparently potassium is essential for growth.

The textbook statement that the animal organism, even in its lowest forms, the protozoa, is satisfied with nothing less complex than glucose as a source of carbon and of protein as a source of nitrogen appears not quite in harmony with the facts.

The Nitrogen-free Diet of Termites.—Another case of an animal which seems able to live for an indefinite period upon a diet lacking in complex nitrogen compounds is the Termite. There are only a few animals which have the power to make use of cellulose (or hemicellulose) as food. Among these are the goat, the cow and the horse, and some insects. L. R. Cleveland has demonstrated that Termites of two genera, Termopsis and Reticulitermes, are able to live and to reproduce regularly and normally upon a diet consisting solely of cellulose. The number of individuals in the colonies increased greatly during a period of 18 months, and the weight of the colonies increased to more than forty times the original weight. The suggestion is made that atmospheric nitrogen is being utilized by the animals for the work of protein building, as this seems to

be about the only possible explanation of their ability to continue living as they do.

Dr. Cleveland also calls attention to a symbiotic relation existing between the Termites and their intestinal protozoa. Termites deprived of their intestinal fauna lose the power to live on cellulose or on wood, while those having their original protozoan intestinal fauna or to which it has been restored are able to digest wood or cellulose and to thrive upon it.

May it not be possible that the apparent utilization of the atmospheric nitrogen by the Termites is really the result of the work of the symbiotic intestinal protozoa, and that they are really doing a work comparable with that of Colpidium referred to above?

Chemical Cycles in Nature.—For ages past men have recognized and emphasized some of the differences which distinguish living from non-living things. Therefore, the *inorganic* world and the *organic* world have come to be discussed. In the one group there are to be found the stable substances of inorganic chemistry; in the other are organisms which are active, dynamic, and which carry on definite metabolic processes. There seemed to the thoughtful men of former days—and to some even now—to be a definite break in the line of continuity between these two groups of substances. At the same time it was recognized that the material substances of the organic realm are derived from the substances of the inorganic realm. It was also recognized that in due time these substances which enter into the composition of the organic beings return to the inorganic realm from which they came and eventually become available for the use of some other organism. It has, therefore, become customary to speak of the cycles through which various substances pass from the inorganic to the organic and back again. Of these cycles the Nitrogen cycle and the Carbon cycle are the most conspicuous. To these should be added the Water, Oxygen, Sulphur, and Phosphorus cycles—less conspicuous but no less important. In time, other cycles may be discussed, as Iron, Iodine, Sodium, Calcium, Potassium, etc., for it is perfectly clear that all of the substances which enter into the composition of the animal or plant substance come from the inorganic environment and ultimately return to it again.

The Water Cycle.—Water constitutes not only a very large and important part of the environment of the lower organisms, and a very constant part of the cellular environment of the higher and more complex organisms, but is a very large and important component of all living protoplasm. The protoplasm of some of the marine forms may contain 95 per cent or more of water. In land animals the percentage of water found in the protoplasm is less, but in all organisms it is an essential part of the colloidal protoplasmic structure. This water, which forms an integral part of the protoplasm, should be distinguished from the water, contained within the organism, which serves simply as a vehicle for the

transport of various chemical substances throughout the plant or animal body. The water within the transport system may or may not become a part of the active, living protoplasm of the cells.

From the lowest animals to the highest there is not only the existence of a high water content, but there is a more or less constant flow of water through the organism. There is an intake of water from the environment, usually into the alimentary system. Some of this water is absorbed and taken into the transport system of the body. From the vessels of the transport system it diffuses into the open tissue spaces and comes to form fo a time the watery cellular environment. It is then absorbed into the living cells and comes to be a part of the proplasmic colloid. Later it is returned to the outside of the cells, back into the transport system and afterwards is eliminated from the body. During its passage inward it may serve as a carrier for food or oxygen; on its way from the cells to the outside it may serve as a carrier for organic wastes. As a part of the protoplasmic structure it is only transitory.

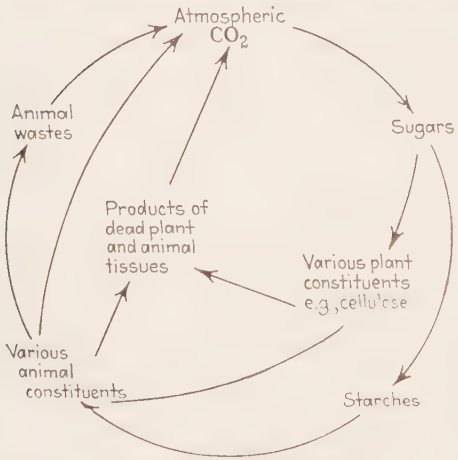


FIG. 52.—The carbon cycle.

The Carbon Cycle.—There is in the earth's atmosphere a small but very constant portion of carbon dioxide. This amounts, over broad land areas, to about four one-hundredths of 1 per cent of the atmosphere. Over the ocean it is somewhat less, due to the absorption of the carbon dioxide by the sea water. This small percentage really amounts in the aggregate to the almost unbelievable amount of 2.5×10^{12} tons in the air surrounding the earth. In addition to the carbon dioxide contained in the air there is an additional amount contained in solution in the waters of rivers, lakes, and oceans, perhaps twenty times as much. Probably this carbon dioxide had its origin in volcanic actions. From these stores in the air and water come all the carbon which is to be found in living matter.

It is not practicable here to enter into a general discussion of carbon exchange upon the earth, but the part of this exchange which has to do with the life processes of animals and plants is of particular interest. There is no other element which shows the same versatility in the formation of compounds as carbon. The number, variety, and complexity of the carbon compounds are unrivaled elsewhere in the whole range of chemical activity.

While the concentration of carbon dioxide in the atmosphere is small, it is sufficient to meet the needs of the green plants. These absorb it from air or water, and under the influence of sunlight, by means of their chlorophyll, build it up into a great variety of substances, such as sugars, starches, fats, oils, etc., or through the activity of protoplasm build it up with nitrogen into proteins. These substances, synthesized by the plants, may be directly oxidized through processes of decay, or through combustion, thus returning carbon dioxide directly to the atmosphere, or they may be consumed by animals, digested, absorbed, and built up into animal substances. These, in turn, will be oxidized and returned to the atmosphere as carbon dioxide. In either case a living organism forms a portion of a cycle involving both organic and inorganic. Of course, it may be said that not all carbon dioxide passes through the cycle, but such as does eventually returns to the atmosphere from which it came. The main features of the carbon cycle are graphically shown in Fig. 52.

The Oxygen Cycle.—Atmospheric air contains approximately 21 per cent of free oxygen. This oxygen is directly related to the carbon dioxide of the atmosphere, and by many authors is considered to have been derived and gradually accumulated from the carbon dioxide through the agency of the green plants in their work of photosynthesis. Such atmospheric oxygen, taken from the air or from solution in water by living organisms, is used in the oxidative processes of the animals and plants and then returned to the environment in combination with carbon as carbon dioxide. Later it is split by green plants, the carbon being used in the synthesis of sugars and starches, and the oxygen being released to the environment where it may be used in the oxidative processes of animals.

The Nitrogen Cycle.—All living things are characterized by the fact that they are largely made up of certain nitrogenous substances known as proteins. Lotka calls attention to the fact that in the human body nitrogen : carbon :: 1 : 3, while in the atmosphere nitrogen : carbon :: 5500 : 1.

Nitrogen is very generally recognized as being an exceedingly inert element. It does not readily enter into combination with other substances. It is very difficult for animals to make use of nitrogen unless it is provided in the form of certain very definite chemical compounds—and nitrogen in such combinations is not overabundant. Plants, especially the green algæ, are better able to utilize nitrogen than are the animals. The amount of nitrogen in the atmosphere, however, which is in a form which can be utilized by plants or animals is generally considered to be very small, probably not more than $\frac{1}{500{,}000}$ of the total amount present. This is in the form of nitrites, which, carried to the earth by rain or in dew, serve to furnish a meager food supply for algæ, and possibly for other

plants as well. It is usually held that the nitrogen of the atmosphere in its molecular form cannot be utilized by plants for food purposes. Some experiments by Benjamin Moore seem to indicate that even such nitrogen may, in the absence of nitrogen compounds, be utilized, even by some of the higher plants, for protein building.

The nitrogen of the atmosphere may be put into usable form for plants by means of the so-called *nitrogen-fixers*. Among the nitrogen-fixers are to be noted an anaerobic bacterium Clostridium, an aerobic form Nitrobacter, and the root nodules or tubercles of the leguminous plants. The value of the root nodules in adding nitrogenous compounds to the soil and in providing such compounds to the plants for protein building depends upon the existence in the nodules of certain forms of bacteria which are really responsible for the work of nitrogen fixation.

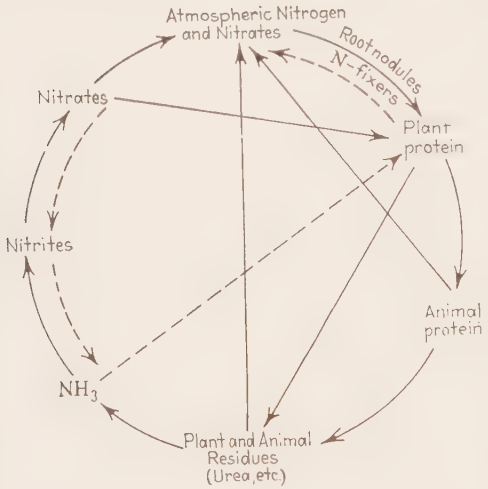


FIG. 53.—The nitrogen cycle.

A glance at the diagram (Fig. 53) will give a picture of the further processes involved in the nitrogen cycle. The nitrogen in usable form, nitrates, or even in the form of ammonia, is built up by the cells of the green plants into plant proteins. These may be eaten by animals and be transformed into animal proteins, which then pass on as nitrogenous wastes, or the plants themselves may suffer decay, and along with the animal residues reach the stage of ammonia compounds. Through the action of Nitrosomonas, the ammonia is converted into nitrites, and through the agency of Nitrobacter the nitrites are changed to nitrates. The nitrates may be utilized as food by plants or through the action of denitrifying bacteria may be returned again to the atmosphere as molecular nitrogen.

It is to be noted that animals have not the same freedom as to the source of their nitrogen as have the plants, but are almost wholly dependent upon proteins which have been elaborated by the plants. It has been shown within recent years that some of the protozoa are able to make use of nitrogen-containing salts in their diet instead of being restricted to the use of the plant proteins. This fact, referred to in another place in this work, serves to break down one of the much-emphasized distinctions between the animal and plant groups.

The Phosphorus Cycle.—One of the most important elements for animal and plant cells is phosphorus. It occurs especially in the nucleoproteins. The actual amount of phosphorus in any living animal is not large; *e.g.*, there is only about 1½ pounds of phosphorus in an adult human being. The phosphorus occurs in the soil in the form of P_2O_5. This is taken up by plants and built into the plant proteins. These, in turn, are consumed and utilized by animals. Or the phosphorus compounds of the soil may be taken by streams to the ocean, which may be considered as a dilute solution of P_2O_5 (0.005 per cent), and thus a nutrient medium for marine algæ and other marine plants. Lindgren has given an interesting paragraph concerning the circulation of phosphorus in nature:

> In the sea water the blue-green algæ concentrate phosphorus, certain molluscs or crustaceans feed on the algæ, and other meat-eating molluscs devour the vegetarians. Small fishes eat the molluscs, large fishes eat the small, finally seals and birds swallow the fishes, and so in about six transformations the phosphorus originally contained in the sea water may come to rest in deposits of guano on desert islands or in accumulations of bones of vertebrate denizens of the sea ("Economic Geology," 1923).

It has further been pointed out that phosphorus becomes increasingly important as animals ascend the evolutionary scale. At any rate, it is very generally true that the higher animals contain larger amounts, relatively, of phosphorus than the lower. This fact may possibly be correlated with a larger proportion of nucleoproteins and of phospholipins in the nervous systems of the higher forms.

Foods.—It is customary to think of food as any substance which is taken into the substance of an organism for the purposes of growth or repair and for the maintenance of the so-called vital processes. The substances employed as food must be of such a character that they may be readily modified to meet the needs of the organism and they must be harmless. Many substances which might be readily absorbed by the organism might prove to be harmful, and many harmless substances might prove to be difficult for the organism to handle. The number and the character of foodstuffs are, therefore, somewhat limited. Also the characters of proper food substances for animals are different from those proper for plants, and even among animals it is found that not all can make use of the same food substances to good advantage.

Brief Classification of Food Substances.—The materials employed by animals are of a rather restricted group and may be classified as follows: (1) inorganic food substances (water, inorganic salts); (2) vitamins; and (3) organic food substances (proteins, carbohydrates, fats, and oils).

Water as Food.—The constant need of every animal body for water is almost self-evident. Water is not alone the vehicle by which all

primary foodstuffs are brought to the cells of the animal body, but the vehicle by which all cell wastes are removed from the cells. It is the agency by which diffusions through the cell membranes take place. No substances can enter the cells or leave them except in the form of dilute aqueous solutions. Also it must be remembered that the living protoplasm is of the nature of a colloidal solution, of which water forms a most important part of one phase. The amount of water held by different tissues varies. Some animals, such as the marine jellyfishes, are almost entirely water, and have been referred to by some authors as *animated sea water*. From such an extreme case, in which the percentage of water entering into the composition of the body may reach 98 per cent of the total body weight, pass to the other extreme, bone, which may contain as much as 40 per cent water. In between these values are found blood (mammalian), containing more than 90 per cent water; liver cells, 75 per cent; nervous tissues, 85 per cent; and muscle, 75 per cent.

Hawk has made a study of the effects of decreased and of increased ingestion of water. When ingestion of water was decreased below a certain minimum in human beings there were headache, loss of appetite, digestive disturbances, and inability to do accurate chemical work. On the other hand, ingestion of nothing but water during a number of days was accompanied by a distinct sharpening of the mental faculties. Of course, such a condition could not continue for more than a relatively short time.

Various chemical processes taking place in the body depend upon the presence of water. In some chemical changes taking place within the body water enters into the reactions. *Hydrolysis* and *dehydrolysis* are terms used to signify the taking up and liberating of water during these reactions.

Hawk illustrates the importance of water in the following paragraph:

> A good illustration of many of the important relationships of water is afforded by the life history of protein, from its ingestion to the excretion of its waste residues. We know that proteins are absolutely essential to the proper nourishment of the human body. In practically all forms in which they are ingested by man they contain *water*. When they reach the stomach they come in contact with the gastric juice, which is 98 per cent *water*. They are there acted on by pepsin, a proteolytic enzyme which causes the protein molecule to *take on water* and break down into substances of a simpler structure than the original protein. These protein cleavage products are then carried into the intestine where the acid of the gastric juice is neutralized by the sodium carbonate present in the aqueous solution we term the pancreatic juice, *water* being formed in the process of neutralization. The enzyme trypsin of the alkaline pancreatic secretion then acts upon the protein cleavage products, *adds water* to their structure, and splits them into still simpler substances. The intestinal juice, which is 98 per cent *water*, now lends its assistance and the enzyme erepsin of this secretion aids

by a *hydrolytic process* in the production of the final cleavage products, the amino acids. These crystalline acids with the help of *water* now pass through the walls of the intestine into the blood, which is over 90 per cent *water*. Borne along by the blood stream these amino acids are quickly transported to the various tissues and organs of the body. Such of the acids as are needed for cell construction are linked together to form the basis of new cell protein, *water* being a by-product of the synthesis. Such of the amino acids as are not needed for this purpose are shorn of their nitrogen by a deamination procedure, the amino acid being thus split into two portions, one nitrogenous, the other carbonaceous. The ammonia formed from the nitrogenous part is passed into the blood, where in the presence of carbonic acid ammonium carbonate is formed. By the removal of *water* from this carbonate we obtain the corresponding carbamate and, by the *removal of water* from the carbamate, urea results. The urea is then removed from the blood by the kidneys and excreted in an *aqueous* solution, the urine. In the meantime the carbonaceous portion of the amino acid has been oxidized in the tissues to yield energy, *water* and carbon dioxide being the final products of this combustion.

Inorganic Salts as Food.—Every organism needs not only the organic materials involved in the building of new protoplasm, but it needs also a variety of purely inorganic substances. The chlorides, sulphates, and phosphates of sodium, potassium, and calcium or the materials out of which these can be made are essential. The cells of the animal body are the descendants of cells which, through long experience, had become adjusted to a certain salt balance in their surrounding medium. The salts serve the purpose of maintaining suitable osmotic relations about the cells, and provide a balanced solution in which the various cells of the body can carry on their reactions in a normal way. As a matter of fact, the normal life of the cell consists not merely in the changes which take place within the cells, but rather of these plus the interaction between the cells and their environment. It is true also that the protein substances entering into the composition of protoplasm are not all soluble in pure water, but in dilute salt solutions. These salts enter definitely into the composition of living matter, and as one bit of living matter is destroyed or made over into something else, new salt supplies are needed for the building of new living substance.

The Vitamins.—The vitamins are substances which must be found in the diets of animals, in order that they may be able to utilize the organic foodstuffs to best advantage. At the present time five different vitamins are known, although only a very few years ago the existence of any such substances was not suspected. These are called:

Vitamin A or fat-soluble A (growth-promoting and antirachitic).
Vitamin B or water-soluble B (growth-promoting and antineuritic).
Vitamin C or water-soluble C (antiscorbutic).
Vitamin D or water-and fat-soluble D (antirachitic).
Vitamin E or fat-soluble E (antisterility).

There is noted in the literature a tendency toward confusion in the nomenclature of these substances.

Nothing is known concerning the part which vitamins may play in the nutrition of invertebrates. In the higher animals, man, mammals, and birds, certain facts are known.

Vitamin A.—This is a growth-promoting vitamin. Animals fed upon a diet deficient in this substance show retarded growth, disease of the eyes, shaggy and unkempt coat, and generally unnourished appearance, and continued failure to secure this vitamin in the food leads eventually to death. The eye diseases and the shaggy coat are caused by the failure of the skin glands to secrete normally. In the eyes, this leads to dryness, bacterial invasion is favored, the eyes become filled with pus, and ultimately blindness occurs. In the skin, the sebaceous glands also fail to secrete normally.

There is some evidence that this substance also plays a part in protecting the body against rickets. It is possible that two substances may be involved, one of which is growth-promoting, and one antirachitic.

Vitamin A occurs normally in many, though not all, animal fats. It is especially abundant in cod-liver oil and in other liver fats, butter, and milk, and in egg yolk. It occurs in oleomargarine in proportion to the amount of fat used in its manufacture and in smaller amounts in oil seeds, carrots, sweet potatoes, yellow corn. This vitamin is not found in most vegetable oils, lard and margarines, white bread, egg white, yeast, purified proteins, and carbohydrates.

Vitamin B.—This water-soluble vitamin is also a growth-promoting substance. Its absence from the diet results in retarded growth ending in death; beri-beri and polyneuritis of birds and mammals are diseases which have as accompaniments inflammation of the nerves, which frequently results in paralysis, partial or total. The presence of this vitamin promotes the normal metabolic processes resulting in growth and prevents the appearance of beri-beri and polyneuritis. This vitamin is especially abundant in seeds, in the germ pericarp and aleurone layer, in yeast, and in egg yolk. It occurs also in smaller amounts in meat, milk, potatoes and some other tubers and roots, in green vegetables, and in fruit juices. It is not found in white bread, polished rice, fats, egg white, purified proteins, or carbohydrates.

Vitamin C.—Almost as far back as written history goes scurvy has been a plague of human life. The cause of the disease was unknown, though some adventurous individuals sought to obviate its destructive power through regulation of diet. Among these was the famous English explorer, Captain Cook. During a sea voyage of 3 years, 1772 to 1775, no case of scurvy appeared in his crew. Dr. Cook assigned as the reason for the continued health of his men the fact that they had been liberally supplied with fresh fruits and vegetables, sauerkraut, and barley. Fol-

lowing his example, it has become common practice for those in charge of the diet of large numbers of men, as in armies, navies, exploring expeditions, etc., to take special precautions to secure an adequate supply of such foods as will prevent the disease.

The fact that the beneficial properties of the various food substances inhere in some definite chemical substance was unknown till about 1919, when the work of a number of investigators led to this conclusion.

Vitamin C occurs abundantly in the juices of the citrus fruits, tomatoes, germinated seeds, cabbages, carrots, beans, apples, turnips, rutabagas, raspberries, liver. It occurs less abundantly in lean meat, oysters, milk, potatoes, many fruit juices, and dried vegetables. It appears to be lacking from seeds, white bread, fats, yeast, purified proteins, and carbohydrates.

Vitamin D.—This vitamin is the antirachitic substance. It may be identical with "Bios," a substance which has been found to promote the growth of the yeast plant, though some investigators believe that this vitamin does not correspond either to vitamin B or to Bios as has been claimed by others. It occurs especially in cod-liver oil.

Lack of this substance during the growing period leads to inability to form a properly calcified skeleton. Rats deprived of vitamin D for 35 to 40 days become unable to use their hind legs. The ends of the long bones of the legs show broad bands of uncalcified cartilage. Adding cod-liver oil to the diet should enable the rats within a period of 2 weeks to start the calcification of the cartilage, and to regain the use of the legs.

It has also been shown that hatchability of fowls' eggs is conditioned by the amount of the antirachitic vitamin contained in the egg. This, in turn, depends upon the amount of ultra-violet radiation to which the fowl has been exposed. It is a rather remarkable fact that ultra-violet radiations have in rickets and tetany effects similar to those obtained by dosing with cod-liver oil—with the exception that the oil works more slowly in the dosage usually given to children. There is still doubt as to the real significance of the similarity of action of ultra-violet radiations and vitamin D.

Vitamin E.—In 1923 it was shown by Evans and Bishop that young rats fed upon a diet containing fats, carbohydrates, pure proteins, salts, and vitamins A and B were incapable of producing viable offspring. They found that it was possible to overcome this sterility by adding to the diet such substances as lettuce, cereals, meat, and egg yolk. Such foods contain a substance different from vitamin C which must be present to ensure a normal pregnancy. They proposed to call this substance *Vitamin X.* As more evidence has accumulated concerning the role played by this substance it has come to be recognized as worthy of a more definite place in scientific literature, and is now referred to as Vitamin E.

CHAPTER XXII

NUTRITION OF DIFFERENT ANIMAL GROUPS

It must be assumed that the student is familiar with the general structure and habits of the forms mentioned. If any doubt exists concerning such matters, a good textbook of zoölogy should be consulted. The limits of the present work are such as to make any discussion of the more common anatomical details impossible.

Nutrition of the Protozoa.—The protozoa are apt to be considered as very primitive organisms, rudimentary ancestors of higher animals, because unicellular. While the higher animals have, no doubt, arisen from simple creatures, it must be maintained that many of the protozoa are now complex, highly differentiated organisms. They are certainly much more differentiated than the earlier, primitive structures which first exhibited the properties of living matter. Of course, there is no definite idea as to the appearance of the first living matter, but it is safe to assume that it did not closely resemble the complex of cytoplasm and nucleus, chromatin, ectoplasm, and entoplasm which is thought of at the present time as the simplest of living forms. It undoubtedly had, too, simpler habits of life than the protozoön of the present day. For example, the Amœba of the present cannot be grown upon a culture medium, unless supplied with bacteria, though dead ones suffice. This means that a supply of protein is essential for the growth of Amœba. Some of the recent work of Peters goes, however, to show that some of the protozoa can be grown in culture media lacking food matter in the form of protein.

The food materials taken by the protozoa are partly of plant and partly of animal origin. Amœba feeds for the most part upon plant food, such as bacteria, algal filaments, etc. The Heliozoa feed only upon animal food. Ciliated protozoa ingest any particles in the liquid surrounding them, without reference to their usability as food. This conception, likely to be gotten by any observer of the behavior of the ciliated protozoa, has been contradicted by Metalnikow, who makes the statement that Paramœcium at least has a certain power of discrimination and of selection of the food particles which it will allow to enter the body.

Food of Rhizopods.—The character of the food of the deep-sea Rhizopods offers some difficulties. It has been held, particularly by some of the English zoölogists, that complex substances of a particulate character would be entirely lacking at the bottom of the deep sea, and

that therefore these animals would be obliged to satisfy themselves with food substances in solution. Thomson expressed the view that as the result of the constant decomposition of plant and animal tissues the sea water of the depths must be of the character of a thin solution of protoplasmic decomposition products. Bütchli, on the other hand, showed that formed decomposition products of the fauna and flora of the coast regions were finally taken to the deep sea, and also that the deep-sea bottom maintains a sufficiently varied animal life of higher organization to maintain through its decomposition products a food supply for such Rhizopods as demand formed nutritive particles.

The process of food taking in other Rhizopods is similar to that of Amœba. The Pseudopods may be of widely different forms, lobose, finely formed threads, or tree-like in appearance. If the nutritive bodies be active infusorians, they stimulate by their contact with the Rhizopod bodies the pouring out of a sticky secretion which is augmented by the added stimulus of attempted escape, so that the potential food particle sticks tighter and is then finally drawn into the protoplasm.

Feeding Experiments upon Protozoa.—Ever since the time of the Count of Gleischen-Russwurm (1777), feeding experiments upon the protozoa have been carried on by many investigators. Any systematic review of the history of the observations upon the feeding of the protozoa would be a long task. A mention of a few of the more important observations may not be out of place. Corti was one of the first to point out the difference in appearance between fasting and well-fed protozoöns, Stylonichia being the example mentioned. It was observed that the fasting individuals had a clearer, less granular appearance.

Ehrenberg showed that when protozoa are fed upon carmine particles these come to lie in food vacuoles. This seemed to him to indicate that the protozoön was equipped with a complete digestive system, or tract, from which the food vacuoles depended like grapes from a stem. The incorrectness of this view was not appreciated as late as 1859, for, in Goadby's "Vegetable and Animal Physiology," on page 98, can be found what purport to be careful sketches of the alimentary systems of Vorticella and Stentor showing the food pouches hanging from the sides of the main alimentary tube. It is now known that this conception of the alimentary system of these forms is not well founded. A fuller account of the formation of the food vacuoles will appear in a later paragraph.

Peptic Digestion of Myxomycetes.—In observations upon Myxomycetes, Krukenberg found that the glycerin extract of certain of the Myxomycetes contained a very powerful peptic ferment, that is, one which has the power to split proteins when in an acid medium. It is possible to extract this ferment from the plasmodium by means of 0.2 per cent HCl, weak vinegar, lactic, acetic, and tartaric acids. He was not able to

account for the presence of a peptic ferment because the protoplasm of the plasmodium always appeared to be alkaline in reaction, and a certain strength of acid is essential for the proper activity of peptic ferments. This difficulty was later cleared up, as will be seen. He noted also that at 40°C. his glycerin extract developed a much more energetic action than at 12 to 20°C. He also found that by heating to 65°C. for 2 hours the extract became inactive. The digestive power of the myxomycete extract appeared to surpass that of some other peptic ferments which Krukenberg secured from invertebrate animals, in that it was capable of digesting not only raw but cooked fibrin to proteoses and peptones.

Food Vacuoles. Reactions.—It was noticed by Celakowsky that the digestive vacuoles of the plasmodium of the Myxomycetes do not always have the same reaction, there being at the same time in the same individuals vacuoles showing an acid reaction and those showing an alkaline reaction. He found also that saturation of the plasmodia with solutions of mineral or organic acids, in such concentrations that they did not interfere with the life of the cells, had no effect upon the rate at which the digestive processes took place, while saturation with sodium carbonate solution of 0.05 per cent strength or with sodium bicarbonate solution of 0.1 per cent had a marked accelerating effect upon the rate of digestion. He observed also that washing out of the alkali by means of water did not counteract the accelerating effect, and hence concluded that the effect of the alkali was not upon the digestive process directly but that the alkali acted as a stimulus for the production of larger amounts of the appropriate digestive enzymes.

Starch Digestion.—There seems to be some difference of opinion as to the degree in which starch granules can be taken up and utilized by myxomycete plasmodia. Krukenberg failed to find any starch-digesting ferment in any of these forms. Du Bary gives an account of the work of Wortman, from which it is learned that starch granules taken up by the protoplasm of Fuligo become strongly corroded during the course of a day. This led him to accept the work of Kühne concerning the presence of diastatic ferments in the protoplasm of some of the lower organisms. He also noted in the plasmodium of Fuligo the presence of a ferment having the power to dissolve cellulose. Celakowsky and also Lister noted that certain of the myxomycete plasmodia were able to digest cooked starch while they failed utterly to digest the uncooked starch. Hartog and Dixon found in the large protozoön Pelomyxa, in addition to a peptic ferment, a diastatic ferment, which would quickly change starch into erythrodextrin, while sugar building was carried on only in a very hesitating fashion.

Hemmeter brought a large mycetozoön, which had taken up a quantity of all kinds of particles from its surroundings, into distilled water which was frequently changed. Gradually the plasmodium freed itself

of the foreign bodies and finally became cleared of algæ, bacteria, infusoria, etc. If these starved protozoöns were then fed with sterile food, such as dried albumin or aleuron grains, it was found that the process of digestion took place so rapidly that there was no evidence of a vacuolar structure, or it was present only to a very slight degree. If the dry albumin was moistened with hay infusion which was rich in bacilli, and was then fed to the protozoön, there resulted, after overfeeding, a condition of rich vacuolization. Hemmeter suggests that the outpouring of an acid liquid into the vacuoles perhaps caused the death of the bacteria which had been ingested, and the disinfection of the food. If this contention should be found to be correct, there would be established an analogy to the assertion that the main function of the hydrochloric acid of the gastric juice is an antiseptic one. The free acids are among the strongest antiseptics known, and it is not at all unreasonable to suppose that these very simple forms of life have found it necessary to defend themselves against the attacks of bacteria which must bring about troublous times if they are not destroyed. The suggestion of Hemmeter fails to take into account the possible function of the acid in transforming zymogens into active enzymes, and also the swelling effect of the dilute acid upon colloidal food materials.

Amœba. Historical Accounts of Feeding.—The Amœba appears to be able to take úp food particles from any part of its surface. The particles come to lie in a vacuole which contains a small amount of water surrounding them. If the animal is dealing with resting or quiet material, only a small amount of water is taken in with the food matter. On the other hand, if the food captured happens to be active a larger quantity of water will be taken into the vacuole. The amount of liquid in the vacuole appears to be accidental and is dependent, to some degree at least, upon the skill with which the prey has been ingested. Sometimes an Amœba will attempt to ingest a large object, such as a Stentor or a Stylonichia. At such a time the Amœba divides itself into two large pseudopods which are brought around the large object in the form of a ring. If the booty escapes before the pseudopodia have united to form a complete ring, they nevertheless continue their movement and come to surround the empty space.

In addition to this type of food taking, in which the animal appears to flow around the food substances, there is another type in which the animal pushes out long, fringe-like, protoplasmic processes which grasp the food matter and draw it in toward the body, which finally engulfs it.

Englemann appears to be the first to record any effect of the protoplasm of the Amœba upon the food matters taken into the body substance. He noted that when an Amœba is fed upon granules of litmus these change in color from blue to red within a few minutes after ingestion. At a somewhat later period Brandt (1881) noticed that in a weak solution of

hæmatoxylin the nucleus of an Amœba took on a pale-violet color, and that the content of the food vacuole, at first colorless, soon became yellowish and then brown in color. This fact he took to indicate the presence in the vacuole of an acid reaction.

Infusorians that happen to be engulfed by an Amœba usually die very quickly, though this is not invariably the case. For example, Jennings cites an observation of an Amœba which captured and engulfed another Amœba, but was unable to hold it. The engulfed Amœba escaped only to be captured again, engulfed, and then escaped. It has appeared to some investigators that there must be something of a poisonous nature secreted by the Amœba during the period of ingestion, of such a nature as to stupefy or kill the ingested material.

If a drop of a very dilute solution of a harmless indicator such as neutral red is added to a slide upon which are mounted Amœbæ in an active, feeding condition, the contents of the various food vacuoles will quickly become stained. The vacuoles will not all be of the same color, some taking a reddish color and others yellowish. Those food vacuoles which have been very recently formed will in nearly all cases show the reddish color indicative of an acid reaction, while the older vacuoles, in which the digestive process may have been going on for some time, will show the yellow color indicating an alkaline reaction. It is doubtful if any real digestion takes place in the vacuole while the reaction is acid. During this time the ingested particles, if living, are killed and either living or dead colloidal elements are materially swollen by the acid and thus rendered more easily subject to the action of the digestive enzymes which will soon enter the vacuole. The acid reaction also serves to activate the enzyme which enters the vacuole in the inactive proenzyme form.

Following the ingestion of the food by the Amœba there is a very quick change in the appearance of the material ingested. If the food matter be of animal origin, the sharpness of outline of food particles is very quickly lost, the particles begin to swell and to become somewhat more transparent, and after a few hours become changed into a granular mass. If the food matter be of plant origin there is a somewhat different history, due to the difference between the animal and the plant cells. The cellulose walls of the plant cells are not affected, but there is an alteration of the protoplasmic structure within the cellulose wall. If the cells contain chlorophyll there will be a change in color from green to brown. Here also there is, as in the animal cells, a transformation of the protoplasmic material. It will be seen that so long as the cell walls of the plant cells maintain their integrity there can be no direct contact of the protoplasm of the Amœba with the food. Digestion, if it takes place at all, must occur by virtue of the fact that the substances concerned in digestion are secreted by the Amœba and then diffused through the cellulose walls of the plant cells.

It has been noted also that there is a difference in the behavior of the food vacuoles which contain indigestible substances as compared with those which contain digestible substances. If indigestible substances are ingested, the water included within the vacuole disappears within a short time. With food vacuoles containing digestible material the case is different. The food does not appear to be dissolved by any direct contact of the protoplasm of the Amœba with the food particles but by a fluid, secreted by the animal, having at the first an acid reaction and diffused from the protoplasm of the Amœba into the cavity of the vacuole.

Tryptic Digestion of Amœba.—Our real understanding of the digestive processes of Amœba and similar forms dates from the work of Greenwood, and Greenwood and Saunders. These authors noted that there is at the beginning of the digestive process, when the food matters are first ingested, a remarkable secretion of acid, and that this secretion ceases with the beginning of the breakdown of the food materials. During most of the course of digestion there is a distinct alkaline reaction in the food vacuoles. Greenwood also noted the fact that the digestive vacuoles move about from place to place in the protoplasm of the Amœba in such a way that there is constantly offered a new protoplasmic surface to the surface of the vacuole. Sometimes granules formed in the cytoplasm become attached to the wall of the vacuole and move along with it. Substances which prove to be indigestible are thrown out after a time. The length of the time during which they are allowed to remain in the body and the place where they are eliminated appear to depend largely upon the amount of inconvenience caused the animal by their presence. Large rotifers and bits of coagulated albumin were observed to be cast out after remaining in the body for 2 or 3 days. Starch was seen to remain for 7 days, and in a very finely divided condition for as long as 12 days.

The assumption of the activity of a tryptic enzyme has certainly been made very plausible through the work of Mouton (1902), who succeeded in extracting from an Amœba an enzyme approaching trypsin in its action. He was able to identify this with the liquid contained in the digestive vacuoles. The enzyme acts in either slightly acid, neutral, or slightly alkaline media, has the power to liquefy gelatine, and is destroyed by heating to 60°C.

Digestion of Starches and Fats.—According to Greenwood and Meissner, the Amœba has great difficulty in digesting starches and fats. At a little later time, Meissner found that if the Amœbæ are deprived of albuminous foods they will break down starches into a substance which give a red color when treated with iodine (erythrodextrin?), and that they finally bring this into solution.

More recent work indicates that the power to digest starch is quite general among the protozoa. The evidence seems to indicate that the

process of digestion by the protozoön is essentially the same as that of starch digestion when treated with diastatic enzymes. It is also a very significant fact that there has been demonstrated in Pelomyxa the storing up of starch and sugar in the form of glycogen during periods of feeding, and the gradual disappearance of it during periods of fasting. The presence of glycogen as a reserve material among the protozoa is a most important contribution to our understanding of their metabolic processes. It is also important that Nierenstein has been able to show, in certain infusorians, that fats may be utilized as foods, stored up, and used as reserve material.

Food Taking and the Nutrition of the Infusoria.—In those protozoan cells which are provided with a firmly formed surface layer the manner of taking up food is, of course, very different from that described for the Rhizopods. In these more highly differentiated protozoan forms there is a small opening, the cell mouth, which leads directly into the thin liquid protoplasm. In these forms the movements of cilia or of flagella serve in the gathering up and ingestion of food particles. As an example, may be mentioned Vorticella. This is a ciliated infusorian whose bell-shaped body rests firmly upon a contractile stalk. At the margin of the disc, where it comes in contact with the side of the bell, there is a spirally arranged band of cilia at the base of which the mouth is formed, and leading for a little way inward is a somewhat funnel-shaped gullet which finally vanishes in the thin liquid endoplasm. The cilia of this band lash rhythmically, and establish in the water a current which is so directed as to bring in small floating particles to the mouth, through which they are forced by the current pressure from the rear and by the contractions of the protoplasm through the gullet and into the endoplasm. In the endoplasm, the ingested particles lie in food vacuoles whose appearance and history are entirely similar to those of the vacuoles of Amœba. There appears to be rather a definite circulation of the food vacuoles through the protoplasm of the cell. The fact that the digestion of the food particles begins and proceeds most rapidly while the vacuoles are lying close to the nucleus has been interpreted by some to mean that the nucleus plays some important rôle in the process of digestion. Any undigested matters contained within the food vacuoles are finally eliminated through the mouth or through the surface of the body at a point near the mouth.

In Paramœcium a definite gullet opening is provided, armed with vibratile cilia, which draw into it food (and other) particles, and after a sufficient mass has been collected these are pressed into the mass of the protoplasm of the body, together with a small amount of water. The particles surrounded by the water which enters with them constitute a food vacuole. The vacuole continues as a definite structure in the cell cytoplasm, and does not mingle with the protoplasm due to the colloidal nature of the latter, and possibly the fact that there may be formed about

the vacuole a definite membrane, which serves as a separation between vacuole and protoplasm.

The protoplasm of the body circulates within the ectosarc and carries the food vacuoles along, thus giving all parts of the body substance an opportunity to pick up food vacuoles. This circulation of the cytoplasm has been carefully studied by Metalnikow.

In Metalnikow's study the animals observed were caused to take up different indicators in powdered form, upon which it was found that the food vacuoles successively presented two different reactions. There is first observed, as in Amœba, an acid reaction in the liquid of the vacuole, which continues for some minutes, and is then replaced by an alkaline reaction which continues until the expulsion of the contents of the vacuole. Metalnikow calls special attention to the succession of the alkaline reaction and points to the very striking analogy which therefore exists between the digestion of the protozoa and the digestion of the vertebrates.

According to the investigations of Nierenstein upon ciliated infusoria it is possible to see in certain endoplasmic granules, stainable by neutral red, an early stage in the production of a proteolytic enzyme. When food matters are ingested these granules collect at the periphery of the vacuoles, then press into the vacuoles, and finally become dissolved in the liquid. Whether events of this sort occur generally throughout the protozoa can only be a conjecture at the present time, but it appears to be highly probable. The fact of the acid secretion first occurring in the food vacuole appears to have its significance in the activation of the zymogens present in the granules which have made their way into the vacuoles.

The dimensions of the vacuoles formed by the activity of the peribuccal cilia vary greatly with conditions. In general, indigestible substances (carmine, sepia, aluminum, etc.) provoke the formation of small vacuoles. Assimilable substances (yolk of egg, albumin) bring about the formation of large vacuoles. As the animal proceeds to nourish itself upon the content of the vacuole the latter becomes reduced in size.

The number of food particles suspended in the water does not appear to influence the formation of vacuoles, though infusoria of the same culture under the same conditions tend to form the same number of food vacuoles. Animals fed upon indigestible substances form fewer vacuoles than those fed upon assimilable substances, and the degree of formation of digestive vacuoles varies in the same individuals at different periods of their activities. When the infusoria are first fed upon a given substance they all form digestive vacuoles in great number. The intensity of formation falls within ½ hour after the beginning of the experiment and later becomes much reduced. This change in rate of formation of the digestive vacuoles is apparently due to the lengthening of the interval between the ingestion of successive vacuoles.

The course traveled over by the vacuoles within the body is almost always the same. The period of circulation varies according to the nature of the substance contained, those containing assimilable substances circulating for a longer time and at a slower rate than those containing indigestible substances.

Paramœcia, according to Metalnikow, are able to choose their food. Animals fed for a long time upon indigestible substances take them in less and less; after a certain time numerous individuals cease entirely to feed upon these substances if they are transferred to a fresh emulsion of them. They will, however, continue to take in other substances in the same manner as before.

If the Paramœcia are fed upon albuminous substances their digestive vacuoles present at first an acid reaction, and following this an alkaline reaction, which continues until the expulsion of the vacuole. In animals which are fed upon fats and carbohydrates, the acid reaction predominates over the alkaline in the digestive vacuole.

Metalnikow's contention that Paramœcium is able to learn to select its food has not been generally accepted. Wladimirsky, in particular, has reinvestigated that part of Metalnikow's work and has come to the conclusion that the latter's results are due to faulty methods of technic.

The Nutrition of the Sponges.—The problem of securing the requisite nourishment is, in the sponge, a twofold one. There is, first, the need of a sessile animal to provide some means of bringing into the animal a continuous current of water which will carry with it substances which may be utilized as food. There is, second, the necessity of meeting the problem of food absorption, after it is once introduced within the chambers of the sponge body.

As has been pointed out, there are on the surface of the animal innumerable pores through which the water may enter. From these it passes through the more solid parts of the body by a series of canals that lead toward a central cavity, the cloaca, finally to emerge through a large opening, the osculum. The osculum is located at the apex of an individual sponge, or, in a colony, each osculum is located upon a slightly elevated portion of the sponge surface.

The current of water is kept in motion by movements of the flagella of what are known as the choanocytes —collar cells —lining many of the canals or specialized chambers in the body of the sponge. The flagella of the choanocytes are constantly active, and are active to such a degree that there is in the cloaca a higher pressure of sea water than outside the body. This pressure varies in different species of sponges, but has been found to vary from 1.3 to 4.0 millimeters of sea-water pressure (Parker, "Elementary Nervous System"). While this pressure seems to be small, it is sufficient to provide for the passage through the canal systems

of the sponges of relatively large amounts of water during the course of a day. Parker has also estimated the amount of water which a colony of Spinosella, having twenty oscula, would pass through itself in the course of a day, and finds it to amount to 1575 liters. Doubtless other observations upon other species of sponges would give similar results.

The nutritive particles made use of by the sponges are very largely the detritus coming from the dead animal and plant tissues found more or less abundantly in the sea water along the coast. From the time of Haeckel, it has been suggested as a possibility that dissolved materials coming from the solution of decomposing animal or plant organisms may also serve as food for sponges.

Nourishment of Aquatic Animals through the Dissolved Organic Matter of Water.—Pütter has put forward the idea that the amount of plankton, etc. eaten by many aquatic animals is insufficient to meet the demands of the organisms, also that the contents of the alimentary canals of animals examined do not indicate a sufficient income of food in the particulate state to support life. He, therefore, assumes the presence in water of dissolved organic matter which may be utilized as food. These organic substances, the carbon-containing substances of the sea, are the products of the metabolism of the marine organisms, especially algæ and bacteria. Pütter believes that he has demonstrated the utilization of dissolved food matters by two types of animals, a sponge, *Suberites domuncula*, and a holothurian, *Cucumaria groubei*.

The carbon exchange of a specimen of Suberites having a weight of 60 grams equals 0.92 milligram per hour. This carbon content may be found in the form of plankton content of the sea water contained in 242 liters, if the figures of Lohman for the plankton content of the water at Syracuse are used. Lohman found 4 milligrams of carbon in the form of plankton in 1000 liters of water. The sponge Suberites must then take in within the space of an hour the plankton from 242 liters of water, an amount equal to 40,000 times its own weight. As a matter of fact, this sponge is able to pump through its canal system only some 300 cubic centimeters of water, $\frac{1}{810}$ of the carbon needed for maintaining its carbon equilibrium. If from the total carbon value of the plankton in the water is subtracted the carbon value of the Copepods, which the sponge is unable to use, it is found that the sponge then gets 0.05 per cent of the carbon supposed to be needed each hour. According to Pütter, the sea water contains 0.92 milligram of carbon in the form of dissolved organic combinations in 14.2 cubic centimeters of sea water. In the 300 cubic centimeters which the animal is able to pass through its canals in the course of an hour, there would be contained 19.5 milligrams of carbon, or twenty-one times as much as the demand. The sponge would need 242 liters if it lived only on plankton, and 14.2 cubic centimeters if it could utilize the organic compounds of the water.

In the same way Pütter has attempted to show that protozoa, medusæ, Anthozoa, Rotifera, Crustacea, Insecta, and Tunicata make use of the materials dissolved in the water. Otherwise there would be necessary the assumption that the animals can make use of the plankton from very large bodies of water. Finally, he has carried the same idea to the nourishment of fishes. One fish studied shows in its intestine, at all times, such an insignificant amount of materials in the course of digestion as to indicate that it could not supply the need of the animal. He says that if it is desired to assert that fishes live only upon formed nourishment, it would mean that the nourishment of the carp is only one-thousandth of that of the perch, since only one-thousandth of the amount of food material is found in the intestine of the carp that there is in the intestine of the latter. There can be no ground for such a supposition, so Pütter concludes that the carp must be able to make use of other material supplies for its needs.

Doubtless inspired by the work of Pütter, other investigators have attacked this very fundamental problem of animal nutrition. Among these must be mentioned Moore, Edie, Whiteley, and Dakin, who have made a very careful investigation of the nutrition and metabolism of marine animals in relationship to dissolved organic matter and to the particulate organic matter of sea water.

There can be little doubt that bacteria are nourished by food matters in solution, nor that bacteria secrete and exude substances which aid in the solution of such organic substances as occur within their spheres of influence. Such dissolved materials are carried away and must give a certain amount of dissolved organic material to the general bulk of the ocean water. The amount of such organic material undoubtedly remains balanced or constant, since it is being broken down into its inorganic constituents as it is oxidized by bacteria or other agencies.

It is entirely possible that many forms of protozoa and other organisms possessing no special alimentary system or free movement of their cytoplasm for the enveloping and digesting of food matters in the particulate form may exist and grow and maintain an oxidizing metabolism on the dissolved organic material so added to the sea, either from the land or from the débris and excreta of the larger and higher denizens of the sea.

It is, therefore, important that something definite should be known of the amount of organic matter dissolved in the water of the sea, so that some idea may be obtained of the value of the sea as a nutrient medium apart from the particulate organic matter in living condition or otherwise which it contains.

Organic matter destined to form some part of an animal body must be broken into smaller molecules and dissolved. These soluble molecules are then built up into the definite colloidal particles which exist in the cell substance of that type of animal. Even in the protozoa, this process

of solution takes place and it is only the soluble molecules which are utilized for the building up of the specific colloidal structures which lie at the basis of species differentiation, and which provide those delicate differences of chemical organization which make possible the physiological differentiations of the tissues of an individual. The same thing is true in the metazoa. Here are provided special cells for elaborating the secretions which are to act as the solvents of organic food materials and break them down into the building stones out of which new organic structure may be built.

It would seem natural, then, that animals should make use of the dissolved organic material in the sea water as a primary source of food. The fact, however, must not be lost sight of that from the protozoa up, almost universally, animals possess mechanisms for making use of food in the particulate form, of dissolving it, absorbing it, and transporting it to all parts of their body structures. And it is to be noted also that as the animal scale is mounted these systems of food preparation and absorption become more and more highly developed—and that the possibility of making use of food matters dissolved in the external medium becomes less and less, until, in the land forms, it has been reduced to an irreducible minimum. This increasing complexity of organ systems for making use of particulate materials would never have reached its present state of development if there had not been a need for it.

If an abundance of dissolved nutrient material existed in the sea, and animals were able directly to utilize it, there would be such a saving of energy that special organs for digestion and for carrying on metabolic work would not develop, and, if developed already, would degenerate.

Sea water would have to be a much richer solution of organic substances than it is in order to support life, having now 0.83 milligram per liter of organic substance—less than one part in a million. Animals living in this fluid would need some substance having a special affinity for dissolved organic matter (as hæmoglobin has for oxygen) in order to supply the tissues of the body with sufficient rapidity. The figures obtained indicate clearly that there is not in the sea water enough dissolved organic material to supply nourishment for any animal.

Similarly, the amount of evenly distributed plankton is far too small for many marine animals to make use of it as a food supply. If the higher animals were able to act as complete filters so that they might collect and utilize all of the particulate matters available, it would be found that they would not be able to filter enough water to supply them with their daily food ration. Sea water filtered through a fine silk net and then through a Chamberland filter yields only about 1 milligram of organic matter per liter, an amount too small to support life. (The figure given is an average for the water of the open sea; of course, there are localities where the figure would be much higher—also localities where it would be much

lower.) Only in sessile organisms with a slow metabolism could the food demand be satisfied by means of the evenly distributed plankton. To this extent do the authors, whose work is here reviewed, agree with Pütter.

According to the work of Moore and his associates, the food supply of these organisms is provided for in the fact that plankton is not equally distributed in the sea water, similar in nature and cause to the unequal distribution of the higher marine animals and of nearly all terrestrial animals. Terrestrial animals have to seek their food, and, as a rule, congregate in considerable numbers where the food is most abundant. If the food desired happens to be motile, they follow the food. Various other biological ends may be served by this aggregating of individuals, but the maintaining of a food supply appears to be the immediate cause of the association of animals. Various animals live together in larger or smaller groups, migrating from one place to another, as the food supply available becomes exhausted or moves away to some new situation. Where there is good pasturage and water, under natural conditions, there will be a greater wealth of herbivorous animals, and where these are found in abundance there will be more of the carnivorous animals which prey upon them. So it happens that some factor in the environment of a permanent physical nature in soil or water, or of seasonal variation, such as light or heat or moisture, produces abundance or scarcity of one species of animal or plant, and this precipitates a whole series of events, the causal relation of which can only be traced and unraveled by most patient study and experimentation.

It has apparently become almost a habit upon the part of many investigators of the floating life of the sea to assume that there is a uniform distribution of the plankton. This assumption is made by Pütter as the basis of all of his work, from which he deduces the insufficiency of the plankton as a food supply, and underlies his argument that the aquatic animals are engaged all the time in exhausting the resources of their immediate environment of plankton and of dissolved organic matter. This leaves out of account anything of the nature of a tropism toward the food in the lower organisms, or of what it has been the custom to consider as instincts among the higher organisms. No selective action of any sort is provided for, and yet it is quite apparent that there is in many organisms a definite response to the food stimulus, of such a character that great aggregations of animals are produced where food conditions may be said to be at an optimum.

Food Taking of Sponges.—The exact method by which the sponge secures its food has for many years been a matter of controversy. In general, the process seems to be now fairly well agreed upon, but there are still details of operation which remain to be investigated. The earlier methods of investigation were doubtless imperfect and so may have led

to faulty conclusions. The more recent work appears to have corrected some of the earlier misapprehensions. As far back as 1856 Carter, and in 1857 Lieberkühn, conducted numerous experiments with a view to finding out how sponges nourished themselves. Haeckel (1872) reviewed their work and came to the conclusion that the flagellated collar cells are the only structures of the sponge having the power to absorb and take up food. He found that these cells fill themselves with carmine grains floating in the sea water, and form them into rather dense masses near the cell nuclei. As to the fate of the ingested particles after they have been taken within the bounds of the flagellated cells there have been a variety of opinions. Keller contended that the food materials are taken over from the flagellated cells by wandering cells of the mesoderm, comparable to the phagocytes of the higher animals, and, as will be seen later, Metchnikoff spoke of the very important rôle of the mesoderm cells in taking up and digesting food materials. Bidder was of the opinion that the flagellated collared cells not only take up the food matters from the water of the canals but also digest that food matter and pass on the products of digestion to the cells in need of nourishment. Mastermann agreed that the flagellated collared cells are the ones to capture and take up the food particles, but held, contrary to the opinions expressed above, that the collar cells themselves undergo physical transformations, losing their flagella and becoming amœboid in character so that they are in no way different from mesoderm cells and that they then wander about from place to place in the sponge parenchyma, where the digestive process is completed. These collar cells which have thus wandered from their original location are then replaced by newly formed elements of the same kind, which themselves ultimately become transformed into mesoderm cells. The amœboid cells, upon the completion of the digestion of the food matters taken in, are supposed to be responsible for the elimination of the remaining waste matters. Vosmaer and Pekelharing are well satisfied that it is the collar cells that are the responsible agents for the capture and ingestion of the food particles. These particles, possibly partly, but certainly not entirely, digested, are passed over to cells in the sponge parenchyma where the digestive process is completed. R. von Lendenfeld, in reviewing this work, also decides that the flagellated collar cells are the cells which take in the food particles, but does not find that these cells sink down into the parenchyma and lose their original flagellated character. Some of the substances ingested, the useless ones, are thrown out by the collar cells; the useful substances are, in a more or less digested condition, given over to the cells of the middle layer, which transport them to such places as may be in need of them.

Intracellular Digestion.—From the statements reviewed above it will be seen that there is no evidence presented of any production by the

cells of the sponges of enzyme-containing secretions, or of the appearance of such secretions in the cloacal cavities of these animals. On the contrary, there is every evidence that digestion among the sponges as well as among the protozoa is carried on in an intracellular manner. Particles are seized by the amœboid processes of certain specialized cells, and then either in these particular cells or in others the process of digestion is completed. The condition appears to be entirely analogous to that existing among the protozoa.

Enzymes of the Sponges.—The solution and assimilation of the solid particles of food material by the cells of the sponges call for the activity of enzymes. After L. Fredericq had shown the presence of tryptic ferments in the parenchyma of some of the sponges, Krukenberg found in the glycerin extract of the parenchyma of different sponges ferments which were capable of digesting raw, but not cooked, fibrin, either in acid or alkaline solution. This evidently indicates the presence of both peptic and tryptic enzymes, though both do not appear to be present in the same form. *Suberites chondrosia*, Geodia, and Hircinia possess peptic enzymes, and Sycon reniera, and Tedania possess tryptic enzymes. These ferments were not found in liquids expressed from the canal systems of the animals, but only in extracts from the cells.

Diastatic ferments capable of changing starch to sugar have been found widely scattered among the sponges, and Cotte has succeeded in identifying in Suberites a fat-splitting ferment, a gelatin-dissolving ferment, and a rennin.

Nutrition among the Cœlenterates.—There is a good deal of difficulty in getting a clear picture of the sequence of events which is involved in the digestion and absorption of food matters by the Cœlenterates. This is partly due to some lack in the number of accurate observations, and partly due to the fact that there has been a modification of the views of physiologists as to the nature of the process involved in this work. The first recorded observations were, apparently, those of Haller, who states that the slimy mass which constantly moistens the walls of the cœlenteric spaces does not show the slightest trace of either an acid or an alkaline reaction, either during the process of digestion or when the organ is empty. Lewes came to the same conclusion and said that the Actinians have no chemical means for the preparation of their food, but that they rather satisfy themselves by mechanically pressing out the sap or juice from the meat taken in. Müller made observations upon crab meat upon which the mesenterial filaments of a living jellyfish had been allowed to rest in sea water. After 10 to 12 hours the meat was found to have been dissolved. Later the Hertwigs made the statement that the mesenterial filaments are secretory organs whose glandular cells have the function of preparing digestive liquids, while the nettle cells serve for the killing of the more or less active animals which are captured and taken in.

Tryptic Digestion of Cœlenterates.—Fredericq was able to demonstrate in some of the Cœlenterates the presence of a tryptic ferment. Following the work of Fredericq, Krukenberg gave special attention to the digestive processes of this group of animals. He fed Actinians with fibrin which was enclosed in small sacs of mull or in quills. The Actinians have the habit of throwing out of the mouth, after a time, all foreign bodies. In order to keep the food within the body, Krukenberg resorted to passing a ligature about the body of the animal just below the tentacles, or fastening the sac in place in the body column by means of a thread attached to the sac and passed through the wall of the foot and fastened to some solid object. The mull sacs and their contained fibrin were weighed before and after their stay in the cœlenteric cavity. The losses in weight were found to be entirely too great to be accounted for on the ground of errors in experimentation. In some cases the fibrin was entirely gone. The slimy mass in the cœlenteric cavity appeared at all times to be incapable of digesting albuminous substances. Even after a lapse of a number of days it was impossible to obtain any evidence of the presence of peptic or tryptic ferment. Upon closer examination it was found that the little mull sacs had been penetrated through and through by the mesenterial filaments, and that digestion had occurred only at those points where the filaments had come into contact with the albuminous food substances. It was, therefore, concluded that digestion was due to the action of the living cells upon the food substances, and not the effect of anything in the nature of a substance secreted by the cells.

It was also found that when fibrin threads were drawn through the side walls or the foot of an Actinian or through the bell of a medusa they were absorbed in from 8 to 14 hours, and this took place while they were in contact with the living tissues. The explanation was offered that the mesenterial filaments force their way into even the most insignificant wounds in the body wall. If access of the mesenterial filaments is made difficult, digestion takes place only so far as the filaments are able to force their way. And it was also found that watery or glycerin extracts of the mesenterial filaments contained substances capable of digesting proteins in the presence of 0.2 per cent HCl. Krukenberg makes the statement that normally the ferment acts only within the cell itself and not in the form of secretion by the cell.

Intracellular Digestion among Cœlenterates.—It is probably true that Allmann was the first to state that intracellular digestion takes place in animals above the protozoa. Before the appearance of the report of the experiments above mentioned, Metchnikoff had made the statement that intracellular digestion is the rule among most of the invertebrates. He said:

> The endoderm cells of the Cœlenterates must be listed in the category of amœboid epithelium. Through the pseudopodia-like processes they take up

the solid nutritive particles after the manner of the Rhizopoda, as is easily seen in the case of transparent forms.

Later Chapeaux followed the intracellular processes among the Siphonophores, whose endodermal cells are capable of taking up particles. He further noticed that granules of blue litmus were taken up by the epithelial cells of the septa as well as by the cells of the mesenterial filament, and became colored red inside the cells. This was taken as an indication of an acid secretion within the cells similar to that found in the protozoans. Chapeaux noticed that fibrin flakes weighing from 2 to 3 grams were entirely digested by an Actinian in the course of 24 hours. The absorption of the food was so complete that it was not possible to detect even a trace of peptone in the gastral space or in the surrounding water. On the other hand, the body substance of a digesting Actinian contains a considerable amount of peptone. In fasting animals only small traces of peptones could be found. If the mesenterial filaments and surface cells of the septa were macerated for some hours in water, an alkaline fluid was obtained which, after filtration, would digest fibrin flakes weighing 2 grams in the course of 8 hours, transforming the whole mass to peptones. If the reaction of the liquid were made slightly acid, the digestion still occurred. In the presence of a stronger acid, such as 1:2500 sulphuric acid, digestion was inhibited. The ferment appears not to be stored up, but to be produced when needed, and was not found at all in the case of fasting Actinians.

From the statements made above, it will be apparent that for the most part digestion among the Cœlenterates is an intracellular process. The endoderm cells chiefly if not entirely responsible for the work of digestion and absorption are amœboid in character, throwing out smaller or larger processes from their free borders as occasion may demand. At times the endoderm cells appear in some cases to fuse together so as to form something of the nature of a syncytium, a mass of protoplasm with several nuclei but lacking in dividing membranes. As a rule, the particles taken into the protoplasm of the cells are of small size, easily to be handled by the pseudopodia of the cells. At other times larger particles than could readily be taken in by a single cell have been seen to be ingested by the protoplasm of several of the fused cells.

Contact Digestion.—The experiments appear to deny the existence of extracellular digestion as a normal process among the Cœlenterates. At least it does not appear to take place to any considerable degree. On the contrary, there are two possible suppositions as to the digestive process. The digestion is either intracellular or what may be termed a *contact digestion*. Among the forms where such structures as mesenterial filaments occur, it is probable that the digestion of such food substances as animals with a chitinous covering is to be explained upon the supposition that the filaments invade the booty of the animal through the natural

openings and penetrate to all parts of the body and there digest and absorb the food matters. At any rate, there is little or no evidence of the free existence of proteolytic enzymes in the gastral cavity.

Friedemann has reached a similar conclusion as to the method of digestion among the Cœlenterates. He says:

> Since a free enzymatic fluid does not exist in the body cavity (of the scyphistoma of *Aurelia aureata*) it is secreted by the single cells as soon as the contact between their surface and a nutritive substance gives the impulse for it.

Gland Cells and Their Functions.—It is still a matter of question as to how large a part may be played in the process of digestion by the uni- and multicellular glands which are scattered about in the endoderm of various Cœlenterates. Certain it is that the endoderm cells of many of the members of the group are not all alike. They appear to be of two rather distinct classes, one definitely glandular, secreting cells, and the other a group of absorbing cells. In the hydroid polyps, the gland cells are widely scattered, but are most numerous in the oral region. In some of the siphnophores, the gland cells well filled with pigment granules form quite dense clusters. They become so massed together as to drive out entirely the absorbing cells from the same region and form glands, as in Vellellen and Porpites. According to Chun, these glandular cells empty their granular secretion into the gastric cavity, where it becomes liquid and evidently has a part in what may be termed the *predigestion* of the food. In the case of a lack of a definitive digestive fluid this production of a secretion could follow only upon the contact stimulation of the gland cells by the object to be digested.

It has also been demonstrated that the isolated mesenterial threads of Actinians and the gastral threads of some of the Medusæ are able, in a watch glass in sea water, to dissolve appreciable amounts of muscle, by contact, and apparently only by contact. It is hard to believe that the process carried out in the watch glass is essentially different from that which takes place normally in the gastral cavity of the living animal.

If such a process of solution as has been described takes place in the gastral cavity, it would mean that there is in that cavity at times a chyme-like fluid. It is also true that in very many of the Cœlenterates the endoderm cells bear cilia or flagella, the movements of which bring about a slow circulation of the liquid contained within the cavity. In forms in which the gastral cavity becomes a much-divided and branched system of tubes, this slow circulation of finely divided, and possibly partially digested, food material becomes very important, as the products of this early digestion may be, and doubtless are, carried to the most distant parts of the body, not by being passed on from cell to cell, but by a sort of circulation, which seems to justify the term *gastrovascular*.

The group appears to present a sort of transition between purely intracellular digestion as it appears among the protozoa and the purely extracellular digestion found in the higher animals. Attention should also be directed to the fact that not only endodermal cells, but mesodermal and even ectodermal cells may take up solid particles from the surrounding medium and digest them intracellularly. In these forms there has not yet occurred that far-reaching differentiation which is to be found in the tissues of the higher animals, and which renders each unfit to do the work ordinarily entrusted to the other.

Digestion among the Alcyonaria.—Pratt finds that large bodies are quickly broken into small particles, and in some cases at least are apparently acted upon by some digestive ferment in the cœlenteron of the zoöids, before being ingested by the cells of the mesenterial filaments.

The mesenterial filaments of hungry zoöids are crowded with gland cells which contain numerous granules. After feeding, the mesenterial filaments contain very few granular gland cells in which the granules are numerous. Many of the cells are entirely empty. The inference drawn is that the gland cells of the recently fed zoöids have poured on the food during its passage through the body cavity and during its envelopment by the filaments a digestive secretion which has brought about its disintegration and partial solution before its ingestion by the cells of the mesenterial filaments.

There is, therefore, evidence in the Alcyonaria (as has also been found among the Madreporaria) of an extracellular or contact digestive process, carried on by the secretion of a digestive fluid in the cœlenteron of the zoöids as well as an intracellular digestion which occurs throughout the whole range of the Cœlenterates.

Digestion among the Worms.—Anything of the nature of a summary presentation of the processes of digestion and nutrition among the worms is attended by much difficulty. The members of the group manifest so great diversity of forms and live under such a variety of conditions as to make it almost impossible to present in a single account any outline of the digestive processes which will apply generally throughout the group.

As might naturally be expected, the Platyhelminthes exhibit a very much simplified digestive process. Some forms have a type of digestion very similar to that which is found among the Cœlenterates; others exhibit a condition not unlike that found among certain of the protozoan forms. The Turbellarian worms are equipped with an œsophageal tube through which food is taken into the inside of the body. This tube opens in some forms into a sort of much-branched or lobed alimentary tract, called an intestine, and in other forms into the intercellular spaces of the body parenchyma, which consists of a more or less definitely aggregated mass of connective-tissue cells, simple or branched, and in some cases apparently fused together into a sort of syncytium with numerous

nuclei. Many of these cells exhibit amœboid movement and appear to exhibit the phenomena common to phagocytic cells, of the blood of higher organisms.

Among the Dendrocœla and Rhabdocœla there occurs a rather well-developed intestine either simple or branched, whose epithelial lining, like that found among some of the Cœlenterates, consists of two kinds of cells, gland cells and ciliated amœboid cells. The latter are especially active in food taking and digesting.

Comparison of Digestion between Worms and Cœlenterates.—As in some of the Cœlenterates, there is to be found among these flatworms evidence of both intra- and extracellular digestion. There appears to be a sort of predigestion of the larger food objects which are taken into the body, and then a passing on of the smaller food particles formed as the result of this process of predigestion from cell to cell, where the digestion is completed in an intracellular manner. In some cases the predigestion appears not to take place in the intestine but rather in the everted pharynx, where it is possible for the secretion of the so-called salivary glands to work effectively. In some of the parasitic forms, such as Distomum, there is evidence of almost typical intracellular digestion. In all of these forms the digestive system functions at the same time as a gastrovascular system in which the nutritive fluid is moved about from place to place by the muscular contractions of the body and by ciliary action.

A similarity of behavior is also exhibited between Cœlenterates and some of the worms through the symbiotic relations which are established between certain members of the group and some of the algæ. This relation appears to be more definitely established and more fundamental in its effects among the flatworms, especially in Convoluta. This form is said to derive its nourishment almost exclusively from the cells of Zoöchlorella which are to be found in its parenchyma.

In 1878, Metchnikoff called attention to the digestion of blood as it takes place in the Turbellarian worms, as being exclusively intracellular in character, taking place in an acid medium, and brought about by the aid of a soluble ferment. It was the earliest proof that typical intracellular digestion is a chemical process, brought about through the intervention of an enzyme.

Digestion in Planaria.—Arnold (1909) again made a study of the digestive process as it takes place in Planaria. He found, lining the digestive cavity, two sorts of cells:

First, long, irregularly columnar cells, the cytoplasm of which consists of a clear protoplasmic network enclosing several large vacuoles at the distal end, the vacuoles toward the middle of the cell being smaller and fewer in number. The proximal part of the cell is a much denser protoplasm and contains the nucleus.

Second, goblet cells. These are one-half or less than half the size of the columnar cells. Each has a small nucleus. The cytoplasm is very granular and stains deeply with basic fuchsin. They are full of large vacuoles. In number they are to the columnar cells as 1:10.

When the intestine of the worm is empty the goblet cells are large and the vacuoles appear to be full. Soon after food is taken into the intestine these cells diminish in size and after 27 hours have shrunken to about one-fifth their original size and are quite flaccid. In this condition these cells are frequently squeezed out of their normal position by other cells lining the intestine. There appears to be no doubt that these cells secrete a digestive ferment, which is probably used entirely in the digestion of fat.

Within a quarter of an hour after feeding, the columnar cells are full of fat globules. No amœboid protrusions from the columnar cells could be seen.

The goblet cells begin to return to normal size after 48 hours, when almost all of the columnar cells are devoid of fat, and become normal in appearance at about 70 hours. No ingestion of solid particles takes place until after fat absorption is completed, and the fat has undergone marked changes in the columnar cells. A large part of the fat absorbed by the columnar cells is digested in the cytoplasm of these cells, but some of it is passed out at their bases, and lies unaltered in the parenchyma.

The first fat appears in the cells in the form of globules, which by fusing together form larger and larger globules, so that in some cases within an hour after feeding some of the cells appear to be solid masses of fat.

Arnold claims that in the Planariæ, as in the higher forms, the neutral fat is split into fatty acid and glycerin by lipolytic enzymes and so absorbed by the columnar cells. The goblet cells appear to act as the secreting organ of the lipolytic enzyme.

It appears, therefore, that digestion in Planaria is not wholly intracellular as has been claimed by Metchnikoff, and really constitutes a sort of first step toward the highly complex digestion found in the higher forms. This first step is the production by certain specialized cells of a secretion which enables the fats to be absorbed. Such cells are unicellular glands. If during the course of evolution these unicellular glands, instead of being diffused, become aggregated in certain areas, it is possible to picture the formation of any of the multicellular glands which arise from the intestinal tract through evagination and enlargement.

Fat globules within the columnar cells undergo marked changes in staining reaction. At first they color a deep black when exposed to the action of osmic acid in the fixing fluid used. Each globule is enclosed in a vacuole. Within ½ hour some of the small globules at the free end of the cell become paler, changing from black to gray and then brown.

Within 2 hours the globules no longer react to osmic acid and the fat takes the less basic of the two stains employed, the fuchsin, until eventually it is stained only by the acid cytoplasmic stain, Orange G. A vacuole is no longer to be seen about the globule, and finally the fat globule is incorporated into the substance of the cytoplasm.

After the fat has all been absorbed, and when all the gland cells are empty, true intracellular digestion begins. The columnar cells push out pseudopodia, which seize and ingest food particles which then appear in large numbers in vacuoles in these cells. These columnar cells appear to have a certain power of selection, for when feeding upon blood they take the red corpuscles first and the leucocytes last (48 hours after feeding). The digestion of the red corpuscles takes place very slowly, and even after 96 hours it is still possible to find some red corpuscles in the vacuoles in the columnar cells. During the process of digestion, the corpuscles lose shape until at last the vacuoles contain only an amorphous mass of particles, consisting chiefly of the remains of the envelopes of the corpuscles.

Arnold calls attention to the interesting fact that, while in the intestine of the animal, leucocytes stain orange by the triple stain (basic fuchsin, methylene blue, and orange G) but after ingestion the cytoplasm of the leucocyte becomes pink, the nuclear content becomes diffused, and shortly afterward the separate nuclear masses are no longer to be seen. This sudden change in the staining reaction would seem to be evidence in favor of the idea that here as in other forms already mentioned the intracellular digestion begins (at least) in an acid medium.

Effects of Parasitism upon Digestion.—The parasite habit is very widespread among the worms, and accompanied by evidences of degeneration in both the digestive apparatus and in the digestive process. Among the Cestodes, the digestive system is entirely degenerated and there is, therefore only an absorption of ready prepared food from the alimentary tracts of the hosts. In these forms there has been lost not only the alimentary tube, but the possibility of producing digestive enzymes.

The Nemathelminthes exhibit an extremely varied degree of degeneration due to their parasitic habit. Almost all members of the group are parasitic, and together they form a most dangerous army of parasites. Some of them are as completely lacking in alimentary organs as the Cestodes. In some the mouth is closed through a proliferation of ectodermal cells. In others an anus is lacking. In still others the intestine shows more or less degeneration. There are also in some cases, in spite of the parasitic habit, well-developed mechanisms for the taking up of food, and a well-developed stomach intestine, but even in these cases it seems to be very likely that the nutritive substances are taken into the body through the skin, and not through the medium of an alimentary system.

The suggestion has been made that the epithelial elements of the muscle pouches are especially active in the assimilation and storage of foodstuffs,

Nirenstein has recently shown that, in the case of Chætogaster, the stomach (probably meaning *crop* or *gizzard*) has an acid reaction equal to that of a N/20 HCl solution. The fluid has no proteolytic action and is probably to be accounted for because of its lethal effect upon the minute organisms which form the natural food supply of the animal.

Functions of the Midgut of Worms.—The midgut in most forms is a straight tube running through the body. In many of the higher forms of worms this is provided with out-pouchings at the side. These serve partly to increase the capacity of the organ and partly for the secretion of digestive fluids and for the absorption of the digested food materials. In many of the higher forms, they appear to be analogous to the so-called livers of the Molluscs and Crustacea. The stomach intestine often shows constrictions at the boundaries of the segments. In the Lumbricidæ the surface of the intestine is increased by the formation of the typhlosole, an infolded portion of the wall. The wall of the intestine generally consists of two coats, an outer muscular coat made up of fibers running longitudinally and in a circular direction, and an epithelial lining, made up of more or less elongated, cylindrically shaped cells whose free ends are supplied with what is known as a striated border, as in Ascaris or Lumbricus. In some cases the cells are also ciliated. In the lower worms the epithelial cells appear to be capable of considerable amœboid movement. Among the higher forms this is not so common, though some observers have thought they found evidence of amœboid activity of the protoplasm of the ends of the cells next to the digestive cavity. In Lumbricus there are retractile cilia or rod-shaped prolongations of the epithelial cells, but it is yet a matter of doubt whether these play any important rôle in the digestion and absorption of food. Two kinds (Fig. 60) of cells making up the epithelial layer are frequently found, one being gland cells often filled with granules representing a digestive secretion, and the other cells which are chiefly absorptive in function. Mention of these will be made in another connection. In the epithelial cells are frequently found enclosures of various sorts, the significance of which is not entirely clear. These enclosures consist of colorless or colored drops, vacuoles of various sizes sometimes filled with a clear liquid and sometimes containing rounded bodies or other kinds of concretions, fat droplets, etc. Occasionally, there are found (not in gland cells) crystalline structures like pigment granules. It is very likely true that these substances mentioned are of the nature of storage materials derived from the intestinal content. It has also been determined that among the Capitellidæ some of the enclosures are substances of a zymogen character, destined to be set free in the intestine for the work of extracellular digestion. Also there is the possibility that some of these substances

may be of an excretory character, as has sometimes been claimed for the enclosures of the chlorogogue cells of the earthworm.

Digestion among the Crustacea.—Not very much is known about the processes of digestion among the lower Crustacea. The midgut of such forms as Daphnia presents a remarkable uniformity of structure throughout its length, and yet there appear to be three distinct regions so far as function is concerned—the absorption of the products of digestion appears to take place in the anterior section, digestion in the middle section, and the formation of fæces in the posterior section. In Daphnia, albuminous bodies, fat drops, unicellular plants, carmine grains, etc., after being swallowed become massed in balls by means of some sticky substance. The insoluble food matters set free in the middle portion of the intestine by the preliminary digestion (fat globules, but not carmine grains) are then forced back into the anterior section of the midgut and reach the openings of the liver diverticula, where they are taken up by the cells. The undigested—and undigestible—are then forced backward by the peristaltic movements of the intestine. If the animals have fed upon green plants there is to be found in the anterior third of the midgut a greenish liquid, which also penetrates into the liver diverticula. The middle third of the midgut contains a dark-green mass of still undigested food material, and the posterior third contains a mass of brownish material, collected to be evacuated. The distribution of the material in the intestine is affected by means of peristaltic and antiperistaltic movements. These movements involve not only the musculature of the intestine but the so-called liver as well. The antiperistaltic movements begin at the boundary between the middle and end gut, and run forward. This wave of constriction pushes ahead of it both dissolved and undissolved substances toward the anterior section of the midgut. The effects of these movements can be very readily observed after feeding Daphnias with egg yolk and carmine. A cohesive mass is quickly formed in the midintestine. As the process of digestion proceeds, fat globules and carmine particles become freed from the mass and then are carried to the anterior section of the intestine, and into the ducts of the hepatopancreatic glands. In a short time it is possible to see the lining cells of the diverticula filled with fat droplets, while the carmine particles are again forced back into the intestine. If the amount of fat taken in is not too great, the fat absorption will be found taking place in the anterior third of the intestine and in the diverticula. If the amount of fat taken into the intestine is larger, it is common to find fat globules in the cells of the middle third also.

The fæces, which are formed in the end gut, are pushed to the outside by peristaltic contractions which have their origin in the midgut.

Among the bees it is found that the enzymes are absent from the crop, the large and the small intestines of the worker and drone, except during

the second half of hibernation. Then catalase appears in the large intestine. In the stomach are to be found catalase, amulase, inulase, lactase, invertase, lipase, pepsin, trypsin, and rennin, but no emulsin.

The Crystalline Style. –A structure which appears in the Mollusca and which has attracted the attention of many investigators is the crystalline style. This structure is located in what may be termed the style sac, a groove on the left side of the anterior portion of the intestine, and is incompletely separated from the intestine by two ridges, the dorsal and the ventral typhlosoles which extend throughout this portion of the intestine.

The style when fully formed fills the lumen of the style sac. It tapers from before backwards and becomes a mere thread at the posterior end of the style sac. The anterior end projects from the pylorus across the stomach cavity to the opposite wall.

Nelson found that the style arises as a thin core of bubbly mucus upon which are deposited coaxial layers of a gelatinous protein containing enzymes. The movement of the style is of great importance in separating the food from foreign particles and in serving as a substitute for peristalsis. The style contains strongly amylolytic enzymes.

A great variety of function has been assigned to the crystalline style. It has been referred to as an organ of support; as an organ in association with the gastric shield, for mastication; as a stirring rod for the stomach content during digestion; as an aid to absorption either by pressing the alimentary matter against the absorbing epithelium or by acting as a plug to prevent too rapid movement of alimentary particles, and to prevent too large particles from entering the intestine; as a reserve of nutriment; as a source of nutriment to useful parasites; as associated in some way with processes of reproduction; as a means of transporting nutriment; as a means of protecting delicate epithelia from abrasion by sand grains, etc.; as a mass of enzymes or enzymes and mucus.

Nelson found that rapid rotation of the style stirs the food mass in the stomach, aiding in separation of food and non-nutrient materials. Lamellibranchs are periodic feeders. The style contains an amylolytic ferment, a reserve for periods of activity. Food particles escaping undigested from the stomach are caught by the cilia of the intestine and returned to the stomach in the core of the style.

Roaf found that the crystalline style of Pecten contains an enzyme which coagulates blood plasma.

Rate of Digestion in Cold-blooded Animals.—The mere fact that different animals are equipped with the same types of catalytic agents cannot be taken as evidence that the digestive processes are likely to go on at the same rates in the different forms, even under similar conditions. Riddle has shown, in a comparative study of the digestive processes in the fish Amia, the frog Rana, the salamander Necturus, and the turtle

Emydoidea, that there is a very considerable variation in the rate of digestion in these forms, being most rapid in the fish and slowest in the turtle. The season is an important factor in modifying the rate of digestion. This may be due simply to the effect of temperature, which has, of course, an important effect upon the rate of these chemical reactions. The minimum temperature at which digestive processes can occur is not the same in the different forms, but is lowest in those forms which hold the lowest position in the animal scale, the order being Amia, Necturus, Rana, turtle. Likewise the maximum temperature at which the digestive processes may go on is highest for those animals which are highest in the animal scale.

Riddle has presented the relative rates of digestion in the forms named in a series of proportions. They are as follows:

Rate of digestion in Amia	:	that in frog	::	7:6
Rate of digestion in Amia	:	that in Necturus	::	17: 8
Rate of digestion in Amia	:	that in turtle	::	29: 14
Rate of digestion in frog	:	that in Necturus	::	31: 16
Rate of digestion in frog	:	that in turtle	::	2: 1
Rate of digestion in Necturus	:	that in turtle	::	117:113

CHAPTER XXIII

ABSORPTION

It has long been a matter of interest to physiologists to discover how substances made up of large molecules are enabled to make their way into the protoplasm of the cells of the organisms. It is, of course, an easy matter to account for the absorption of substances of low molecular weight, but it is not easy to account for the absorption of the substances which do not readily go into solution, or whose molecules are so large as to prevent them from passing through membranes with ease.

Possible Methods of Fat Absorption.—A number of suggestions have been made as to fat absorption by animals. Fatty matters in large masses are not able to pass through cell membranes with any appreciable rapidity. There is no certainty that the same method applies in all cases, and it may be true that even in the same animal more than one method may be employed at different times In general, there seem to be the following possibilities:

1. The fat may be absorbed in the form of minute droplets which are broken off from the fatty masses in the alimentary tract, or which may be separated from the fat masses by the activity of the absorbing cells.

2. The fat may be digested in part by lipase, forming fatty acids and glycerin. This fatty acid may then assist in emulsifying the remaining fat, and the fine emulsion thus formed may be absorbed by the epithelial cells of the alimentary tract. The manner in which the minute fat droplets get into the cells has not been explained, though attempts have been made to show that the cells possess amœboid properties. The suggestion has also been made that the cilia which are in many cases found upon the free borders of the absorbing cells may play an active part in the fat absorption.

3. There is the possibility that the fats may be absorbed in the form of fatty acid and glycerin, resulting from the digestion of the neutral fat by the lipase.

4. According to the solution theories, the fat may be absorbed in the form of soaps. These theories assume that the neutral fats are split into fatty acids and glycerin by the lipase found in the pancreatic or other digestive juice; that the fatty acids unite with the alkali of the intestinal secretions to form soaps; that these soaps are soluble in water, and are, along with the glycerin, absorbed into the epithelial cells and there resynthesized into fat.

In favor of the theory that the fat is absorbed in the form of soap it may be said that it has been shown that alkaline soaps can be absorbed, and that a mixture of soap and glycerin can be synthesized into fat within the cells. It is also true that soaps are found in the intestine only in small amounts, thus indicating that soap absorption is rapid. Dissolved fatty acids may be absorbed in small amounts. The fatty acids are practically insoluble in water but are slightly soluble in bile.

Among the objections to the soap-absorption theories are the facts that the reaction of the intestinal content during absorption is acid, and that it is impossible for soaps to exist in an acid medium. This might be overcome by saying that the acidity of the intestinal content is due to the presence of organic acids, coming probably from the split fats, and that these do not act in the decomposition of soaps.

It has been suggested that an enormous amount of alkali would be required to unite with the ordinary amounts of fatty acids present, but this theory loses weight when the process of resynthesis is considered. As soon as the soaps reach or enter the epithelial cells the fatty acid is at once liberated and reacts with the glycerin in the formation of neutral fat, and the whole of the alkali is set free to be used again in saponification of a new supply of fatty acid.

Brief Historical Statement.—A very brief summary of the history of our knowledge of fat absorption may be of interest. The first histological demonstration of the absorption of fat by the intestinal epithelium was by Webber in 1860. Just a few years previously von Kölliker had shown that fat may be absorbed by the stomach in the dog, cat, and mouse. In 1902, Schilling was able to show the presence of fat droplets in the epithelial cells of the gastric mucosa of the calf. Van Herwerden in 1908 showed that following feeding with fat there is an abundance of fat droplets in the lining epithelium of fishes. Greene in 1912 showed that the king salmon is able to absorb fat from all parts of the alimentary tract, and that this is the prime function of the pyloric cœca. In the same year Greene and Skaer demonstrated the absorption of fat in the stomach of the dog, cat, and rat. It has also been found in the author's laboratory that it is possible for the earthworm, the salamander Diemyctylus, and the frog to digest undivided fat and to absorb it from all regions of the intestine. Sanford has shown essentially the same for the cockroach. It may therefore be stated that fat absorption is a possibility in all classes of animals above the worms.

As far back as 1838, Marcet showed that when dogs are fed upon cooked meats and sheep fat and the content of the intestine examined after 4 or 5 hours a considerable amount of fatty acid is always to be found. Cash in 1860 also found that there is an increase in the amount of fatty acid present when neutral fats were subjected to the action of extracts of the gastric mucosa in the presence of hydrochloric acid. Both

of these observations add weight to the suggestion that lipase is to be found in the gastric secretion and that there is an active splitting of the fat into fatty acid and glycerin.

Brucke was responsible for the idea that fat is absorbed in the particulate form. Since his work was published, others have given attention

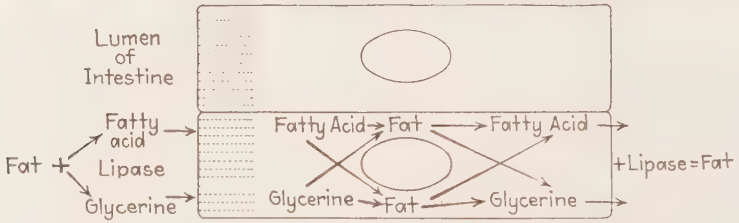


FIG. 54.—Diagram to illustrate fat digestion and absorption.

to the manner in which the fat is absorbed. Krehl, in 1890, found that fat is absorbed in the dissolved condition, and then resynthesized within the epithelial cells. Rachford, in 1891, found that the pancreatic juice is capable of hydrolyzing all the fat of an ordinary meal in the normal period of pancreatic digestion. Monk holds that the fat is absorbed in

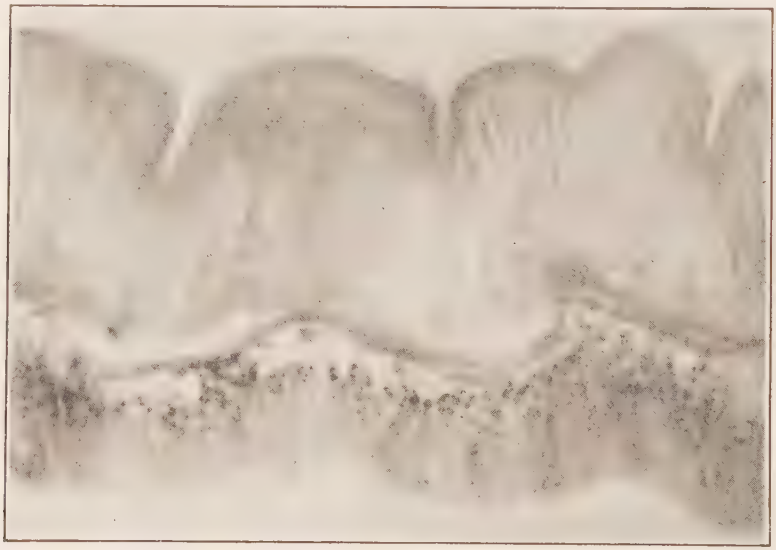


FIG. 55.—Lining epithelium of the stomach intestine of the earthworm, unstained, containing no fat. This worm had been starved for a few days, being killed and sectioned.

the form of fatty acid and glycerin. Moore and Rockwood in 1897 came to the conclusion that fats are absorbed both as fatty acids dissolved in bile and as soaps. If the reaction of the intestinal content is alkaline, the absorption is believed to be as soaps; if it is acid, both methods may be employed. They do not believe that fats are absorbed in the form

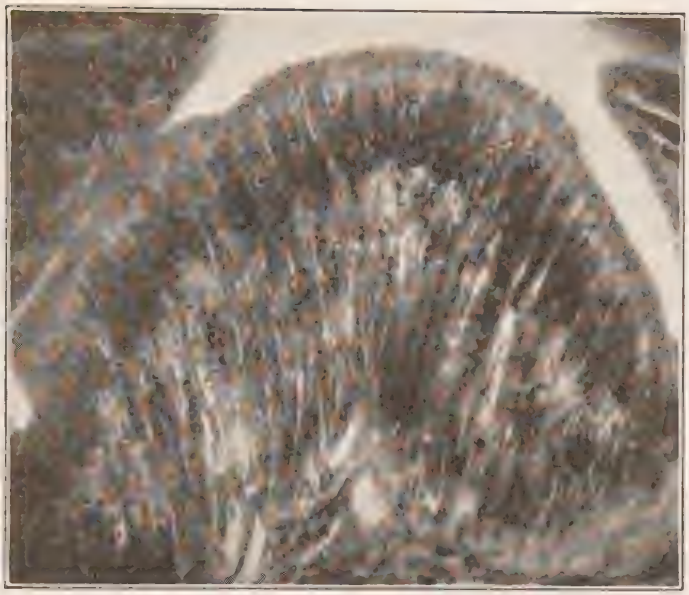


FIG. 56.—Lining epithelium of the stomach intestine of the earthworm after feeding with olive oil. Fat droplets shown in black in the outer ends of cells, but never in the striated border. Little fat in proximal ends of cells. Preparation fixed in Flemming's solution, not stained.

FIG. 57.—Lining epithelium of the stomach intestine of the earthworm, showing a condition of approximately maximum fat absorption. Technic same as in preceding figure.

of emulsions, but think that emulsifying aids in the absorption. In 1898, Cunningham put forward the idea that oil in the finely divided or emulsion condition is more readily absorbed than when not so divided. This is because of the much greater surface of contact. Whitehead in 1909 and Plant in 1908 had both come to the conclusion that fat is absorbed from solution as soap and not from an emulsion. Hawk also has accepted this idea.

Of course, the really great advance in our understanding of this whole matter came from the investigations of Kastle and Loevenhart, who worked out the idea of the reversible action of lipase. According to this theory the fat will be first acted upon by the lipase and split into fatty acid

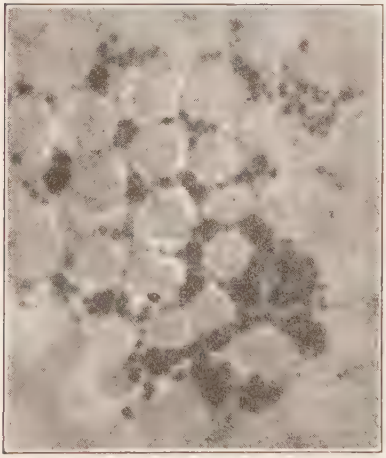


Fig. 58.—Section cut transversely to the long axis of the epithelium cells lining the stomach intestine of the earthworm. Note that fat absorption occurs in certain cells only, while others, the secreting cells, contain no fat.

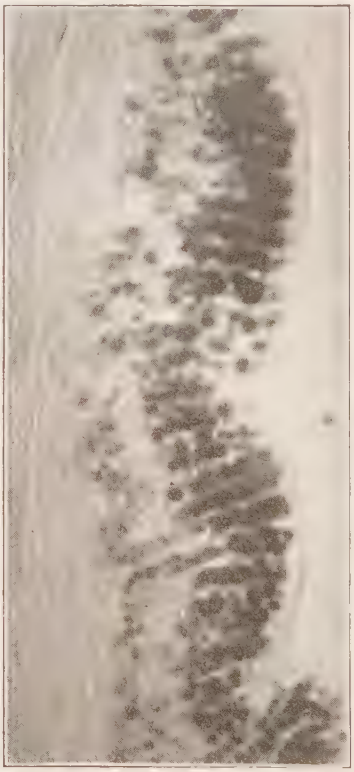


Fig. 59.—Photograph of the intestinal epithelium of the frog *Rana palustris*, showing a high degree of fat absorption.

and glycerin. If the fat is emulsified by the action of bile, the action becomes the more rapid. The fatty acid and the glycerin may then diffuse through the exposed border of the absorbing cells, or the fatty acid may enter into combination with the alkali in the lumen of the intestine to form soap, in which case the soap is free to diffuse into the cell. Within the cells the soap may be broken down and the fatty acid liberated. The liberated fatty acid or the acid which has diffused into the cell is then ready to enter into combination with the glycerin which has also diffused into the cell. This action takes place within the cell

under the influence of lipase. Before the fat is again to leave the cell there must be another splitting of the fat into fatty acid and glycerin. Figure 54 indicates in a graphic way some of the possibilities of the case.

Experiments made in the author's laboratory by some of his students indicate that fat absorption occurs very readily in the stomach intestine of the earthworm, and in various portions of the intestines of the frog and salamander. In Fig. 55 is shown a section of the wall of the stomach intestine of an earthworm which had been starved for several days. The preparation was fixed in Flemming's solution, sectioned and mounted unstained. Note that the epithelium cells lining the digestive cavity are devoid of any fat droplets. In Fig. 56 is shown a similar preparation of the stomach intestine of an earthworm which had been fed upon olive

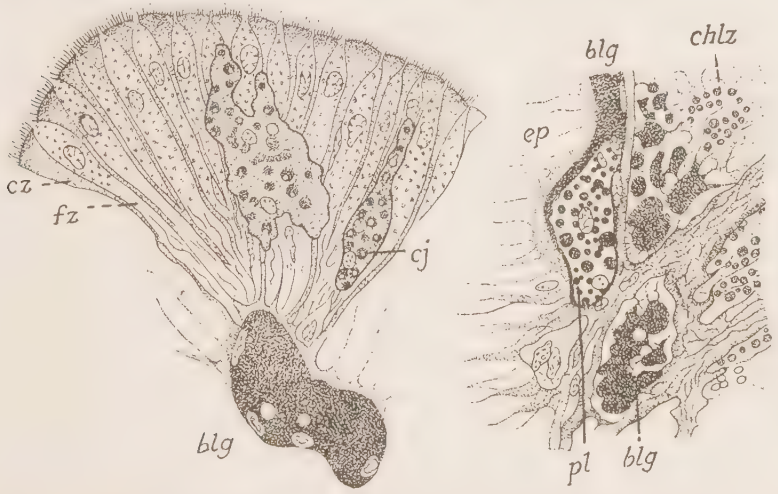


FIG. 60.—A piece of cross-section through the stomach intestine of *Lumbricus*. *blg*, blood vessel; *chlz*, chlorogogue cells; *ep*, intestinal epithelium; *cz*, ciliated cell, fat absorptive; *fz*, secretory epithelial cells; *cj*, yellow cells; *pl*, plasmodium in an intestinal vessel.

oil some 12 hours before being killed. Note the presence of the blackened fat droplets in the outer ends of the epithelium cells. Figure 57 represents the condition of the fat-absorbing cells of the earthworm about 24 hours after feeding with olive oil. Not all of the epithelium cells are involved in fat absorption. This fact is made evident by a glance at Fig. 58, which shows the epithelium cells of the stomach intestine of the earthworm cut across their long axis. The absorbing cells are filled with the blackened droplets of olive oil. In between these cells are the enzymes of importance to the earthworm. In Fig. 60 is shown a sketch of a bit of the lining epithelium of the stomach intestine of lumbricus, indicating the existence of at least three sorts of cells in this layer. Similar absorptive conditions may also be demonstrated in the intestinal epithelium cells of vertebrates, as is shown in Fig. 59. This is from a

section of the intestine of a frog which was killed a number of hours after feeding with olive oil. The fact of absorption is shown here as clearly as in the earthworm. It should be noted that in no case is there any indication of fat droplets in the borders of the cells, or in the very outermost layer of cytoplasm of these absorbing cells.

Absorption of Carbohydrates and Proteins.—It is a matter of regret that there have not been developed methods by which it might be possible to trace directly the absorption of the products of carbohydrate and of protein digestion. It is known that the carbohydrates generally are broken down into the simple sugars, dextrose and levulose, in the alimentary tracts of animals, and that these same sugars occur in the circulating blood. It is also true that the sugars occur in greater concentration in the blood just coming from the intestine than in blood in other regions of the animal body. It is also known that proteins are broken into amino acids in the course of digestion, and methods have been devised by which it is possible to detect the presence of amino acids in the blood stream.

Place of Absorption.—Among the lower metazoa absorption of food appears to occur chiefly in the lower or posterior portion of the alimentary canal, and in some cases through the linings of the so-called livers, or digestive glands. Among the higher animals absorption occurs only rarely through the epithelial linings of the oesophagus or stomach, nearly all food materials and water being absorbed through the lining epithelium of the intestine. Sugar appears to diffuse directly from the lumen of the intestine, through the epithelium cells covering the villi or folds of the small intestine, into the blood as it circulates through the capillaries of the mucous membrane. The prevailing idea concerning the absorption of the products of protein digestion is that the amino acids coming from the digestion of the protein food diffuse directly through the epithelium lining the wall of the intestine into the blood stream. Some small portion of the amino acids may diffuse into the lymphatics and thus reach the blood in an indirect manner. From this blood—which after the digestion of a meal is a solution of various amino acids, sugars, and fats—the tissue cells select the particular elements needed for their constructive, repair, or other work.

Method of Absorption.—Various authors have found it impossible to harmonize the absorption of food substances with the generally accepted notions concerning osmosis. They have, therefore, postulated a *selective action* upon the part of the absorbing cells. Such selective action would, of course, involve the expenditure of energy upon the part of the absorbing cells—and such expenditures of energy have not as yet been clearly demonstrated.

Experiments have shown that equimolecular solutions of different substances are absorbed from isolated loops of intestine at different

rates. This fact appears to be in harmony with observations made upon the relative rates of diffusion of different substances through parchment and similar membranes. In the case of non-living membranes difference of rate of diffusion is interpreted as indicating difference in molecular size, or difference in the solubility of the diffusing substance in the substance of the membrane, or difference in the diffusion pressure of the substances in solution. In every case diffusion appears to occur in the direction of from higher to lower concentration. The diffusions which occur in the absorption of food materials appear, therefore, not to be different in kind from those which take place in the processes of respiration. Nor do they seem to be of a different sort from those which occur in the laboratory through parchment membranes.

CHAPTER XXIV

UTILIZATION OF FOOD MATERIALS IN THE BODY

A statement of the needs of every animal body for materials to repair the worn-out protoplasm, to build new protoplasm, and to supply energy to run the body machine has already been presented. It has been pointed out that animals, in general, require highly complex substances as the basis of the food supply and, in particular, substances which contain nitrogen, sulphur, and phosphorus. These are the proteins. To these should be added the carbohydrates and the fats, organic substances which can supply heat energy but which cannot supply the needed nitrogen.

As the result of digestive processes there comes to be in the alimentary tract a solution containing not only proteins, starches, disaccharids, and fats, but also the products of the digestion of their substances, *viz.*, amino acids, simple sugars, fatty acids, and glycerin. These substances are all of relatively low molecular weight and diffuse readily through the membranes of the epithelium cells which line the alimentary tract. They soon appear in the circulating fluids of the body.

Among the higher animals the amino acids are probably absorbed directly into the blood capillaries, and are not rebuilt into proteins in the intestinal epithelium. The sugars also diffuse directly into the blood stream. The fatty acids and glycerin, on the other hand, diffuse into the lining epithelium cells and are there rebuilt into fat droplets. Sooner or later they are again split into fatty acids and glycerin and pass into the lacteals or lymph spaces, where they are recombined into fat.

Factors Determining Value of Protein Food. – The value of protein as food depends in part upon digestibility. Many proteins, particularly those of animal origin, may be very completely digested. Some, like the proteins of egg white, are relatively indigestible Egg white in its most favorable condition is about 58 per cent digestible; raw, it is much less digestible. Proteins of beans and peas range from 70 to 80 per cent digestibility; those of apples, prunes, and strawberries are extremely indigestible. These differences in digestibility are in part due to the presence in them of indigestible carbohydrates.

For a long time it was supposed that protein building takes place in the epithelium cells of the alimentary tract. The chief reason for this belief was the lack of suitable methods for detecting the presence of amino acids in the blood. Such methods have now been devised and

there is very good evidence in favor of the transportation of amino acids as such in the blood stream. As a part of this evidence there should be mentioned the work of Buglia (1912), who found that it was possible to inject into the veins sufficient amounts of amino acids to meet the nitrogen food requirements of the animal body.

There has already been given a list of the amino acids which have been found to enter into the structure of protein molecules (see page 275). Out of these primary building blocks are built all the various proteins. In Table 45 is shown the percentage composition of some forty-six different protein substances, the analyses of which are more or less complete. There is still some doubt as to the exact number of molecules of each amino acid which enters into the composition of each of these proteins and of their arrangement in the protein molecule. The evidence given in this table indicates the very great differences in the structure of these particular proteins. There can be no question that the proteins of different animals represent different combinations of these amino acids. It is probably equally true that in a single animal the proteins of the different tissues are just as truly different as are those of different species. If the proteins of every species of animal are in some respect different from those of every other there must be an almost unlimited number of forms of protein molecules developed in different animal tissues. Of course, the total number of compounds which can be formed from the nineteen different amino acids is very great.

Adequate and Inadequate Protein Foods.—For the building of any particular protein certain definite amino acids are necessary. Different proteins call for different combinations of amino acids. For example, tryptophane is an amino acid which is found entering into the composition of many proteins. If the diet of the animal fails to contain tryptophane it will be impossible for the animal to construct the essential protein. The same would be true for any essential part of any other protein. The animal cannot build its proteins unless it has the proper building blocks. For example, Osborne and Mendel found that the protein Zein of maise, which is lacking in tryptophane, lysine, and cystine, is an inadequate food for rats. Gliadin, found in wheat and rye, and hordein, found in barley, are proteins lacking in lysine. They permit the maintenance of body structure but do not provide for growth. Casein, edestin, gluten, lactalbumin, and vitelline are, on the other hand, adequate for maintenance and growth.

Some amino acids, such as glycine, may be manufactured within the animal body. This would make possible the use of food lacking in glycine for protein building, even though glycine is needed for the protein of the body. Tryptophane, lysine, and tyrosine cannot be so manufactured in the bodies of higher animals, and must therefore be components of the food proteins taken in. Some proteins are better foodstuffs than

TABLE 45

	Monoaminomonocarboxylic acids									Monoaminodicarboxylic acids		Diaminomonocarboxylic acids		Diaminooxymonocarboxylic acids	Heterocyclic compounds				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Glycine	Alanine	Valine	Leucine	Isoleucine	Phenylalanine	Tyrosine	Serine	Cystine	Aspartic acid	Glutamic acid	Arginine	Lysine	Cascinic acid	Histidine	Proline	Oxyproline	Tryptophane	Ammonia
Protamines:																			
Salmine			4.3					7.8				87.4	0		0	11.0			
Sturine												58.2	12.0		12.9				
Clupeine		+	+					+				82.2	0		0	+			
Scombrine													0		0			+	
Cyclopterine						8.3						62.5	0		0			+	
Cyprinine I						+ ?						4.9	28.8		0				
Cyprinine II			+			+						+	+		0				
Histones:																			
Globin from Hb of horse blood		4.19		29.04		4.24	1.33	0.56	0.31	4.43	1.73	5.42	4.28		10.96	2.34	1.04	+	
Globin from Hb of dog blood		3.0	1.0	17.5		5.0				2.5	1.73					4.5		+	
Thymus histone	0.5	3.5		11.8		2.2	5.2			0	0.5	15.5	6.9		1.5	1.5			
Lota histone												12.0	3.2		2.9				0.7
Albumins:																			
Egg albumin	0	2.1		6.1		4.4	1.1		0.3	1.5	8.0					2.3		+	
Serum albumin		2.7	0.9	19.4		2.4	0.9			1.0	10.1					4.0			
Bence-Jones albumin	1.7	4.5		10.6		1.5	1.7			4.5	6.0					1.9			
Globulins:																			
Serum globulin	3.5	2.2	+	18.7		3.8	2.5		0.7	2.5	8.5					2.8		+	
Fibrin	3.0	3.6	1.0	15.0		2.5	3.5	0.8	1.1	2.0	10.4					3.6		+	
Edestin (hemp seed)	3.8	3.6	+	20.9		2.4	2.1	0.4	0.3	4.5	6.3	11.7	1.0		1.1	1.7	2.0	+	
Glutelins:																			
Legumin (pea)	0.38	2.08		8.0		3.75	1.55	0.53		5.30	13.80	10.12	4.29		2.42	3.22		+	1.99
Amandin (almond)	0.51	1.40	0.16	4.45		2.53	1.12	?		5.42	23.11	11.85	0.70		1.58	2.44		+	3.70

Glycinin (soy bean)	0.97	.....	0.68	8.45	.....	3.86	1.86	.....	.....	3.89	19.46	5.12	2.71	....	1.39	3.78	.....	.....	2.56
Phaseolin (white kidney bean)	0.6	.....	1.1	9.7	.....	3.3	2.2	0.4	.....	5.3	14.6	4.9	4.0	....	2.0	2.8	.....	.....	2.1
Protein of maize	0.3	.....	.....	6.2	.....	1.8	3.8	.....	.....	0.7	12.7	7.1	3.0	....	3.0	5.0	.....	+	2.1
Protein (fir-tree seed)	0.6	1.8	+	6.2	.....	1.2	1.7	0.1	0.3	1.8	7.8	10.9	0.3	....	0.7	2.8	.....	+	
Conglutin (lupine seed)	0.8	2.5	1.1	6.8	.....	3.1	2.1	+	.....	3.0	19.5	6.9	2.1	....	0.7	2.6	.....	+	
Avenin (oats)	1.0	2.5	1.8	15.0	.....	3.2	1.5	.....	.....	4.0	18.4	.....	.....	....	...	5.4			
Gluten (wheat)	0.4	0.3	.....	4.1	.....	1.0	1.9	.....	.....	0.7	24.0	4.4	2.2	....	1.2	4.0	.....	+	2.5
Legumin (bean)	1.0	2.8	1.0	8.2	....	2.0	2.8	.....	.....	4.0	16.3	4.6	5.1	....	1.1	2.3			
Gliadins:																			
Gliadin (wheat)	0.8	2.7	0.4	6.0	.....	2.6	2.4	0.2	.....	1.3	31.5	2.8	0	....	1.2	2.4	.....	1.0	4.1
Gliadin (rye)	0.13	1.33	.....	6.3	.....	2.7	1.19	0.06	.....	0.25	33.81	2.22	0	...	0.39	9.82	.....	+	5.11
Hordein (barley)	0 0	0.43	0.13	5.67	.....	5.03	1.67	.....	.....	.....	36.35	2.16	0	...	1.28	13.73	.....	+	4.87
Zein (maize)	0.0	2.23	0.29	18.6	.....	4.87	3.55	0.57	0	1.41	18.28	1.16	0	....	0.43	6.53	.....	0	3.61
Phosphoproteins:																			
Caseinogen (cow's milk)	0	0.9	1.0	10.5	.....	3.2	4.5	0.23	0.06	1.2	11.0	4.84	5.8	0.75	2.59	3.1	0.25	1.5	1.6
Caseinogen (goat's milk)	0	1.5	.....	7.4	.....	2.75	4.95	.....	.....	1.2	12.0	.....	.....	....	.....	4.6	.....	+	
Caseinogen (human milk)	0	...	...	...	...	...	4.71												
Vitellin	1.1	+	2.4	11.0	.....	2.8	1.6	−	.....	0.5	12.2	.....	.....	....	.....	3.3			
Scleroproteins:																			
Gelatine	16.5	0.8	1.0	2.1	−	0.4	0	0.4	−	0.6	0.9	7.6	2.8	....	0.4	5.2	3.0	0	0.4
Silk gelatine	0.2	5.0	.....	.....	.....	.....	5.0	6.6	.....	.....	.....	+	.....	....	4.0				
Silk fibroin	36.0	21.0	.....	1.5	.....	1.5	10.5	1.6	.....	.....	0	1.0	+	....	.....	+			
Spider silk fibroin	35.2	23.4	.....	1.8	.....	.....	8.2	.....	.....	.....	11.7		5.24		.....	3.7	.....	.....	1.2
Elastin	25.8	6 6	1.0	2.14	....	3.9	0.4	...	.....	+	0.8	0.3	...	....	....	1.7			
Spongin	13.9	.....	.....	7.4	.....	.....	0	.....	.....	4.7	18.1	.....	.....	....	.....	6.3			
Egg membrane (Scyllium)	2.6	3.2	.....	5.8	.....	3.3	10.6	.....	.....	2.3	7.2	3.2	3.7	....	.....	4.4	.....	+	
Keratin (ox horn)	0.4	1.2	5.7	18.3	.....	3.0	4.6	0.7	6.8	2.5	3.0	2.3	.....	....	.....	3.6			
Protein derivatives:																			
Syntonin	0.5	4.0	0.9	7.8	....	2.5	2.2	.....	.....	0.5	13.6	5.1	3.3	....	2.7	3.3	.....	.....	0.9
Heteroalbumose	...	...	.....	....	...	.....	.....	.....	.....	...	...	8.5	7.1	.	0.4	...	....	....	1.0
Protalbumose	...	...	.....	...	...	...	.....	.....	.....	.....	..	4.6	3.1	...	3.4	..	...	.....	0.8

others because of the particular combinations of amino acids which they bring to the body tissues.

Some proteins contain amino acids which the animal does not need for building its own proteins. Other protein foods lack amino acids which the animal must have. These facts make it clear that not all proteins have the same biological value.

Some proteins contain fragments which are digested with very great difficulty; others contain substances which exert an inhibitive effect upon proteolytic action. In some cases the digestion of a certain protein in the diet is conditioned by the presence of another protein.

The net protein value of a protein food may be calculated as a function of the percentage of the proteins in the food substance, the percentage of digestibility, and the percentage of availability (the percentage that can be used to meet the protein requirement at ordinary levels of protein intake). Mitchell cites two cases illustrating this point: Beef contains 20 per cent protein, of which 95 per cent, or 19 per cent of the total, is digestible. If only 80 per cent of this can be used by the body, the net available protein is 15.2 per cent. Navy beans contain 22 per cent protein, of which 80 per cent, or 17.6 per cent of the the original, is digestible. Since only 38 per cent of this amount is available for body purposes, the net is 6.69 per cent.

In general, animal proteins serve better than plant proteins. This difference in nutritive value was set forth by Karl Thomas, who took starch and sugar in large quantity in his diet, determined the minimal loss of body protein under these circumstances, and then added to the diet food materials containing different proteins, in order to determine their relative power in sparing the body from a loss of tissue protein. The values given below Thomas named the *biologic values* of the proteins employed.

Table 46.—Biologic Values of Different Proteins, as Measured by the Percentage Quantity of Body Protein Which Their Ingestion Will Spare from Loss

Ox meat	104	Yeast	71
Cow's milk	100	Casein	70
Fish	95	Nutrose	69
Rice	88	Spinach	64
Cauliflower	84	Peas	56
Crab meat	79	Wheat flour	40
Potatoes	79	Cornmeal	30
Cherry juice	79		

Amino acids not used to build proteins can be used for energy. While the full history is not known, it is known that deaminization occurs. This means that an NH_2 group is split off to form ammonia, and that this is later changed to urea and eliminated. The part of the amino acid remain-

ing may be oxidized to furnish energy, or built into sugar or fat and oxidized at some later time. The amino acids may thus serve to supply energy just as sugar or fat.

Among mammals, deaminization occurs in the liver, though this may not be the only place in the body where it can occur.

The amino acids which are most likely to serve as a source of sugar in the body are glycine, alamine, aspartic acid, glutamic acid, proline, arginine. Leucine, lysine, and tryptophane do not serve as a source of sugar.

In every organism, two sets of processes take place side by side, anabolic or constructive and catabolic or destructive. During early life, every individual shows an excess of anabolic processes, as is evidenced by increase of body mass and weight. This occurs notwithstanding the fact that the youthful individual is, on the whole, more active than the older of the same kind. During middle life anabolic and catabolic processes are nearly equal in amount. During later life the catabolic processes are in excess, and are indicated by loss of weight of body tissue, frequently resulting in actual shrinkage of body.

Each animal apparently attempts to maintain an equilibrium between income and outgo along several different lines, namely, nitrogen, carbon, and water.

Nitrogen Equilibrium.—By *nitrogen equilibrium* is meant the fact that the animal body in a series of days tends to excrete as much nitrogen in urine, perspiration, etc., as it takes in as food. Nitrogen storage in the animal body does not apparently take place, in the same sense as does carbohydrate storage. Such storage of nitrogen as does take place is for the great part in the form of new body protein.

Carbon Equilibrium.—By carbon equilibrium is meant the tendency of the animal to maintain an approximate balance between the amount of carbon taken in as food and the amount of food given off from the body as carbon dioxide. It is apparent that if more carbon is taken into the animal body than is consumed, there is some form of storage within the body. On the other hand, if the amount of carbon given off as carbon dioxide is in excess of the amount taken in with the food, some carbon compounds contained within the body must be consumed.

Carbohydrates and fats are food substances which do not contain nitrogen. They are inadequate, therefore, for the building of protein. They have high fuel value, and so are able to serve for the production of heat, and as a source of energy for muscular work.

When carbohydrate or fat is taken in as food it is used to conserve the body proteins which might otherwise be used in furnishing the energy needed for various metabolic activities. Any excess of carbohydrate or of fat above the amount needed to supply the energy for muscular work may be stored within the animal body.

Complete Balance Experiments.—No complete understanding of the metabolism of an animal body can be attained without making complete balance experiments. In these experiments the intake of oxygen and of carbohydrate and nitrogenous foods is accurately measured, also the outgo of carbon dioxide, of nitrogenous waste in the form of urine and fæces, and of heat is carefully determined. The first experiments of this sort were made by Voit and Pettenkofer. They did not attempt to deter-

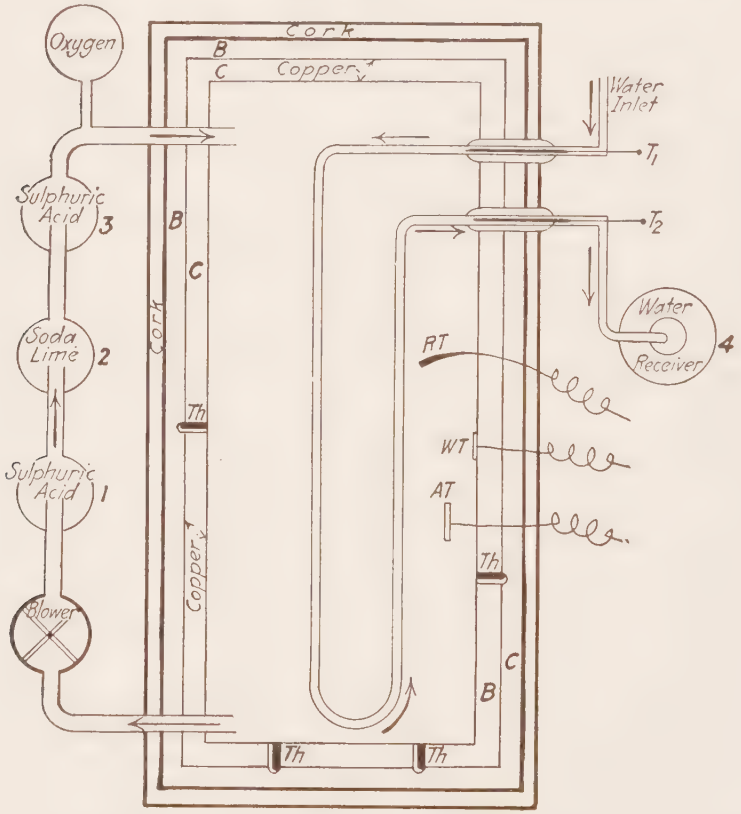


FIG. 61.—Diagrammatic plan of respiration calorimeter, Atwater-Rosa-Benedict type. Absorbers 1 and 3 to take up water vapor, absorber 2 for carbon dioxide; *B* and *C*, dead-air spaces; *Th*, thermocouple; T_1 and T_2, thermometers for water as it enters and leaves the chamber; *RT*, rectal thermometer; *WT*, wall thermometer; *AT*, air thermometer. (*After Lusk.*)

mine the heat production of the animal experimented upon, but only the use of oxygen, the carbon dioxide produced, and the nitrogen intake and outgo. This was made possible through the use of a specially constructed apparatus known as respiration chamber. The development of the more accurate calorimetric methods now in use for determining metabolism of animals and particularly of human beings is due to the work of Atwater

and Rosa, Rubner, Benedict, Williams, and others, who modified the respiration chamber into a respiration calorimeter.

In the earlier work, a stream of fresh air was kept passing through the respiration chambers. At intervals samples of the outgoing air were taken and analyzed and an estimate made of the total amount of carbon dioxide produced. In more recent forms of the apparatus (Fig. 61) all of the carbon dioxide is collected as carbonate by soda lime, and new oxygen is admitted to replace that which has been consumed. In this way, the determination of the oxygen used and the carbon doxide given off can be used to check each other.

It is well known that nitrogen forms about 16 per cent of most proteins. This amount varies somewhat, but is sufficiently accurate for all except the most exact determinations. If, then, the amount of nitrogen found by analysis of urine and fæces be multiplied by the factor 6.25, the total amount of protein broken down in the body will be found.

The general results of this type of work are the following:

1. Protein is essential for maintaining body structure.

2. Each organism establishes for itself a level of nitrogen metabolism which is modified only with difficulty. If more than the needed amount of protein is taken in the excess, nitrogen will appear in the urine. If less nitrogen is taken in than is needed, there is a loss of protein material from the body.

3. Protein food when taken in excess may be deaminized and used to furnish energy for bodily activity.

4. Proteins which in themselves may be inadequate for the upbuilding of body protein because of the lack of some specific amino acids may, notwithstanding this fact, serve as tissue sparers.

5. A diet containing carbohydrates and fats serves to protect body proteins. The non-protein foods may be increased and the nitrogenous food decreased to a marked degree without interfering with nitrogen equilibrium.

Carbohydrate Metabolism of Animals.—Sugars and starches are for the most part the products of the photosynthesis carried on by green plants. It has already been pointed out that these substances have great energy value, although they do not provide for the rebuilding of animal tissues.

The extensive use of carbohydrates by higher animals is itself an evidence of evolution. There is little evidence indicating the use of starches or sugars by the protozoa. Apparently no one has found among the protozoa enzymes capable of digesting these substances. Among the Porifera and Cœlenterata, however, and in all higher groups such enzymes are found. In the lower forms the enzymes are of a rather generalized type, while in the higher forms they are more highly specialized.

It might be said that the development of muscular activity has been dependent upon the ability of the organism to metabolize sugar. Certainly, the high efficiency of the wing muscles of insects and of the muscles of birds and mammals is associated with a highly efficient use of sugar. Animals fed upon a wholly protein or protein-and-fat diet may continue to do muscular work. This does not mean that the protein or fat is being directly utilized by the muscle, but probably does involve the transformation of some part of the protein or of the fat into carbohydrate, which is then used by the muscle.

Glycogen Formation and Use.—Our present understanding of the utilization of sugar in the animal body goes back to the work of Claude Bernard, who demonstrated that, following the ingestion and digestion of carbohydrate foods, there is an increase of sugar (glucose) in the blood of the portal vein. The excess of sugar above the normal for arterial blood is removed from the blood stream by the liver cells and stored as *glycogen*. It also happens that muscle fibers have the power to store glycogen. These storages must be brought about through the activity of an enzyme—glycogenase. As the concentration of sugar of the blood is reduced below the normal, there is a splitting of the glycogen of the liver (or of muscle), and a restoration of the normal concentration of sugar (glucose).

Blood Sugar.—The carbohydrates which are most commonly ingested by animals consist of a variety of sugars of the aldohexose and ketohexose types, such as glucose, fructose, mannose, and galactose, having the formula $C_6H_{12}O_6$; aldopentoses ($C_5H_{10}O_5$); disaccharids, such as saccharose, maltose, and lactose, having the formula $C_{12}H_{22}O_{11}$; the polysaccharids, glycogen; the starches, dextrins, celluloses, inulin, lichenin, all of which have the formula $(C_6H_{10}O_5)_n$; the hemicelluloses, agar-agar, and gum arabic, whose chemical formulas are unknown. All of these except glucose are acted upon by the enzymes of the alimentary tract and are split or transformed into glucose, in which form they are absorbed. Other sugars may possibly be absorbed into the blood stream, but for some reason the cells of the body are probably not able to utilize them, and if absorbed they will be excreted unchanged. Excretion of glucose occurs in the human being when its concentration exceeds 0.16 per cent in the blood. There is little information as to the normal concentration of glucose in the bloods of the lower animals.

Occurrence of Glycogen in Lower Animals.—Glycogen was discovered at about the same time by Claude Bernard and Hensen, and indications are that it is found in all cells that have the possibility of growth. Glycogen, or some substance similar to it, occurs in many of the lower animals. Paramylum, a starch-like substance ($(C_6H_{10}O_5)_n$) occurs in the flagellate Euglena. Kühne found glycogen in protoplasm of the Myxomycete Æthalium and claimed for it identity with the glycogen

of liver and of embryonic muscle. A substance very similar to glycogen has been found by others in Paramœcium, Opalina, Bursaria, and Vorticella.

Another carbohydrate, paraglycogen (also referred to as zoöamylum), occurs abundantly in the Gregarines. None of these substances has been found among the Porifera. How these animals and echinoderms store up carbohydrates is uncertain, though Picard claims to have found glycogen in all of them. Among the worms, Lumbricus, Tænia, and Ascaris, glycogen appears. In the last-named form it makes up as much as 2 to 7 per cent of body weight. In some cases it is reported that almost one-half of the living substance is glycogen. Intestinal worms are proof against action of diastase as well as proteolytic ferments. Question may be raised as to the presence of antienzymes. It is known that among the Mollusca glycogen reaches its highest concentration—and, possibly, its highest importance. It is found in Cardium to the extent of 14 per cent; oyster, 9.5 per cent; Pecten muscle, 2 to 2.5 per cent; foot of Helix, 3 to 3.5 per cent of body weight.

Among Arthropods large masses of glycogen occur in fly larvæ, but no sugar. Sugar appears only in the pupa stage. A single example must suffice. Intensive investigations in regard to carbohydrate metabolism of the insects were made by Bataillon and Couvreur. They found that the content in glycogen and sugar during metamorphosis of the silkworm goes through a remarkable change. A one-day pupa contains about twice the amount of glycogen found in the silkworm at the time that it begins to spin itself in. The maximum of glycogen storage is reached during a life section in which the animal takes no nourishment; therefore, glycogen must arise at the cost of body substance. Parallel estimates of the content of glycogen and fat show that the maximum of the one accompanies the minimum of the other. Glycogen seems also to arise at the cost of fat. The stored-up glycogen during the latter section of the pupa stage goes over into sugar; so that at the end of the stage the sugar content reaches its maximum, whereas the glycogen for the most part has vanished. At the time of emergence, the entire carbohydrate store is in the form of diffusable sugar, circulating in the liquid medium, easily consumed.

These facts indicate that the carbohydrate metabolism has many analogies with that of the higher animals, and that here as there the carbohydrate reserves are partly found in certain organs in the form of glycogen or in a substance closely related to it in order to serve as energy sources in the case of need.

Functions of Sugar in Animal Body.—While sugar does not supply the needed nitrogen for tissue building, it does, because of its abundance in the diet and the ease with which it is oxidized, play a most important part in the economy of the animal.

1. It supplies the energy needed for muscular work and for other work upon the part of the cells of the body.

2. The combustion of sugar supplies the heat needed for maintaining the body temperatures of all homoiothermous animals. Each gram of sugar yields upon combustion 4 calories of heat. The heat energy given off by muscle during contraction and relaxation is far in excess of the energy used in the work accomplished by the muscle.

3. The protein of the body is protected by the use of sugar as a source of energy.

4. If there be an excess of sugar over the amount used by the body as a supply of energy, it may be stored as glycogen -or it may be synthesized into fat.

When sugar alone is burned in the body as the source of energy, the respiratory quotient = 1.

Utilization of Fats in the Body.—Excess of fat or of carbohydrate food taken into the animal body may be stored as fat in special adipose cells. These are especially abundant in the subcutaneous tissues, in the folds of the peritoneum, etc. If there is a lack of sufficient sugar or sugar-forming substances in the body to furnish the energy for muscular work or to maintain body temperature, the fat supplies of the body are called upon. The fat is oxidized, as would be sugar, into carbon dioxide and water—or may in certain cases be transformed into sugar before it is consumed. Fat yields 9 calories of heat per gram instead of the 4 calories yielded by sugar.

Animal Heat.—As the results of the work of many investigators, it has become commonly recognized that, in so far as regulation of body temperature is concerned, animals may be divided into two great groups. These have been referred to by John Hunter and others as the cold-blooded and the warm-blooded animals. It has long been recognized that the first of these groups includes animals whose temperature changes with the temperature of the environment, while the other includes animals which maintain at all times a practically constant body temperature. In order to give recognition to this fact, Bergmann suggested the term *poikilothermous* to describe those animals with variable temperatures, and the term *homoiothermous* to refer to those animals whose temperature is maintained at more or less constant values in all atmospheres.

The earlier work upon temperature determinations was done by the use of mercurial thermometers; the conditions under which many of the observations were made were not all that could be desired; and the results obtained were far from satisfactory. The following table taken from a paper published by the author and one of his students illustrates the great variation in the results obtained from observations made in this rather crudely conceived way.

From these data it might be surmised that many of the animals have some form of temperature-regulating device, very imperfect perhaps, but

which suffices to maintain a body temperature slightly above that of the surroundings.

TABLE 47.—BODY TEMPERATURE OF CERTAIN COLD-BLOODED ANIMALS

Form	Range	Conditions and method	Temperature of animal above that of environment	Observer
Medusa	.....	.....	0.27°F.	Valentin
Earthworm	56°F.	Several in glass by thermometer	2.0–2.5°F.	Hunter
Leeches	56–54°F.	Several in glass by thermometer	1.0–1.5°F.	Hunter
Worms	.....	.....	4.4–5.8°F.	Davy
Ceylon jungle leech	.....	.....	Same as water or air	Davy
Echinoderms	.....	.....	0.40°F.	Valentin
Snails	54°F.	Several in glass by thermometer	1.25°F.	Hunter
Snail	76.25°F.	.....	0.25°F.	Davy
Oyster	82°	.....	Same as water	Davy
Crustacea	72–80°F.	.....	1° below to same as water	Davy
Insects	62–83°F.	.....	8° below to 10.5°F. above	Davy
Bees	19.2°C.	Individuals—thermoelectric	0.18°C.	Dutrochet
Bees	.....	Hive	0.25°C.	Dutrochet
Bees	.....	.....	0.55–10.5°C.	
Bees	57–69°F.	Thermoelectric	Same as air	Phillips and Demuth
Bees	Below 57°	Thermoelectric	Form clusters and raise temperature	Phillips and Demuth
Fishes:				
Bonita	80.5–78°F.	Heart muscle	1.5–5°F.	Davy
Trout	86 40°F.	.....	28° below to 2°F. above	Davy
Eel	51°F.	.....	Same as air	Davy
Fishes(?)	.....	.....	0.55°F.	Martin
Fishes(?)	.....	.....	1.38°F.	Kraft
Fishes(?)	.....	.....	6.2–9.3°F.	Broussouet
Fishes(?)	.....	.....	1.2–6.2°F.	Broussouet
Carp	.....	.....	3°F.	Boniva
Carp	.....	.....	0.86°F.	Depretz
Carp	66.5°F.	Stomach	1.9–3.5°F.	Hunter
Mackerel	80.5°F.	Stomach	8.5°F.	Davy
Herring	4.6–6.04°C.	.....	0.0 1.2°C.	Simpson
Amphibia	58–86°F.	Rectal temperature	3.0° below to 8.5°F. above	Davy
Frog	.....	In air	Temperature less than air	Berthold
Frog	.....	In water	Same as water	Berthold

Poikilothermous Animals.—As a matter of fact, all of the invertebrated animals and the lower vertebrates belong in the group of animals of changeable temperatures. It was the privilege of the author with one of his students to test the validity of the published statements with methods somewhat more refined than had been used previously in regard to a few selected forms chosen from different animal groups. The body temperatures of the earthworm, clam, salamander, and goldfish, as

compared with that of rather rapidly circulating water which formed the environment, were determined by thermoelectric methods. In every case it was found that the temperature of the animal, when changed from water of one temperature to that of a different temperature, assumed the temperature of the new environment to within a few hundredths—usually to within a few thousandths—of a degree within a very short time. In the earthworm, this time was as short as 2 minutes. In the clam, weighing with its shell about ½ pound, the time was longer than in any of the other forms, ranging from ½ to ¾ hour. This was probably due to the mass of material involved and not to any heat-regulating activity. The final temperature of the animal as recorded was found as likely to be slightly below the temperature of the surrounding water as above it. No evidence whatever was found for the existence of any heat-regulating apparatus in the forms studied—the earthworm, freshwater clam, salamander, and goldfish.

Homoiothermous Animals.—The body temperature of the homoiothermous animals is the result of the balancing of a number of different processes. Heat is the result of oxidation and other chemical processes of the body. Heat is given off during muscular contraction, and more especially during the recovery phase which occurs during the relaxation of the muscle. Of course, the heart muscle which is always at work produces a very considerable amount of heat. The chemical processes involved in digestion in the alimentary canal are heat producing, as are the chemical changes taking place in the various glands of the body, particularly the liver. This gland, because of its size and the part which it plays in carbohydrate metabolism, is the center of much heat production. Some authors have contended that the nervous system, and particularly the brain, is a center of heat production. It is true that the nervous system is well supplied with blood vessels for carrying to it an abundant supply of oxygen, but it has yet to be demonstrated that any unusual amount of heat is produced as the result of nervous activity. The rapidity at which all these various processes take place, *i.e.*, the rate of metabolism, then, determines the amount of heat produced.

Variations of Temperature of Man and Other Animals.—It is a common phenomenon that the temperatures of even the homoiothermous animals are subject to much variation. In some animals, such as the cat, dog, and rabbit, the heat-regulating mechanism is not operative at birth. The young animals are practically poikilothermic at birth and continue to be so for some days, though it may be said that their body temperatures will not approach quite so closely to that of their environment as will the body temperatures of the permanently cold-blooded animals.

The homoiothermic condition of the higher animals is maintained in part through the activities of the nervous system. When the nervous

mechanism involved in heat regulation is disturbed in its operation through the use of anæsthetics or through disease the animal becomes more or less poikilothermic in behavior. This was demonstrated by Wakelin Barratt in rabbits suffering from rabies, and by Simpson in a monkey under deep ether anæsthesia. Accompanying the lowering of body temperature nearly to that of the surroundings there is a slowing of heart action and of respiratory movements. These actions simply follow what is known to be the usual order in case of physiological operations dependent upon chemical reactions.

Daily variations of temperature have been observed in man and other higher mammals. In man it is generally true that the body temperature is at its minimum during rest and sleep between 2 and 6 a.m. During the hours of activity and labor the temperature rises from the minimum of, say, 36.3 to 37.5° between 11 a.m. and 6 p.m. and then slowly falls again. It is stated that when the daily routine is revised so that the period of sleep comes during the day the daily temperature change is also revised. It is also reported that the body temperature of nocturnal birds is highest during the night.

Why is the Homoiothermous Condition Desirable?—The body of every animal is a great laboratory in which a great variety of chemical operations must take place at very definite rates in order that the animal body shall carry on all its different operations in a normal and well-balanced order. It so happens that different chemical operations are differently influenced by temperature. For some of the operations which occur in the body, the optimum temperature may be 40°, for others 50°, for others some other temperature. The highest temperature at which the various chemical reactions may occur is also a variable. Because of this differential effect of temperature upon the chemical reactions of the animal body, it follows that some nearly constant temperature provides a condition in which the animal may the more readily maintain a proper balance between the different essential reactions. As the temperature of the animal departs from the normal, some operations are bound to be retarded, and others accelerated, relatively, with the result that the metabolism of the body as a whole is deranged.

To balance this heat production there is a heat loss through the skin, lungs, urine, and fæces. The loss through the skin may be through conduction, or radiation, or through the evaporation of moisture from the surface. The loss through the lungs is through the air and water vapor which is warmed while in the air passages. Urine and fæces are eliminated from the body as substances heated to body temperature, and this heat is thus lost to the body. In fur-bearing animals, heat loss through the skin is difficult, as is shown in the dog and rabbit.

Heat Production and Heat Loss.—The body temperature of an homoiothermous animal represents a balance between heat production and

heat loss. This balance is maintained through the interaction of a number of factors, some of which are chemical, some physical. In general, it may be stated that the physiological operations leading to heat production are chemical; those leading to heat dissipation are physical.

Factors Influencing Heat Production.—Heat production is for the most part the result of oxidations taking place in all parts of the animal body and is more rapid in younger than in older animals; in smaller than in larger (per unit of body mass); and in females than in males. In this work of heat production the muscles are especially involved, though some glands, such as the liver, play an important part. Heat is also liberated by the processes of digestion in the alimentary tract. Very little heat, relatively, is liberated by the elements of the nervous system.

The rate of metabolism and therefore heat production of the poikilothermous animals are very definitely limited by, and proportional to, the body temperature. The homoiothermous animals, on the other hand, are obliged to maintain a nearly constant temperature. If the temperature of the environment falls, there will be greater heat loss from the body, and to compensate for this there must be a more active metabolism. This may be brought about through the stimulation of receptors sensitive to lowered temperature, and the reflex stimulation of muscles, bringing on shivering movements.

The following data of Rubner's quoted from Lusk indicate clearly the effect of external temperature upon the metabolism of a fasting guinea pig.

TABLE 48

Temperature of air, degrees Centigrade	Temperature of animal, degrees Centigrade	Grams of CO_2 eliminated per hour and per kilogram of animal, degrees Centigrade
0.0	37.0	2.905
11.1	37.2	2.151
20.8	37.4	1.766
25.7	37.0	1.540
30.3	37.7	1.317
34.9	38.2	1.273
40.0	39.5	1.454

Specific Dynamic Action of Protein Food. According to Rubner, the intake of protein food serves as a distinct stimulus to increase the general metabolism of the body. In certain cases a larger diet of meat may cause an increase in the total metabolic rate of as much as 90 per cent. The effect of carbohydrate food is much less, amounting to 30 to 40 per cent.

Heat Centers and Heat Nerves.—At various times it has been suggested by physiologists that the bodies of homoiothermous animals have an equipment of *heat centers* and *heat nerves*. These are supposed to

affect directly the heat-producing tissues, and to be entirely distinct from the motor or nerves that influence the functional activities of the tissues. It must be confessed that the evidence for the existence of these nerves is hardly conclusive. Ott has shown that in the rabbit there is a spot in the *corpus striatum*, the puncture of which leads to a marked increase of heat production, although there may be no other apparent effect from the operation. Heat centers have been located in the cortex, midbrain, pons, and medulla by other observers. Barbour found that there is a spot in the *corpus striatum* which has regulative powers upon heat production. When this spot is warmed to 42° by a special device, there is a fall of body temperature, and when it is cooled to 33° there follows a distinct rise in body temperature.

Body-mass and Body-surface Factors in Temperature Regulation.—Rubner has shown that it is necessary to distinguish between the body mass and the active protoplasmic mass of an animal when considering its normal metabolism. Every animal contains some non-living material. In the protozoön this may be in the form of granules or crystals within the cells. In the metazoan animals this non-living material takes the form of inert substances used for binding various parts of the animal body together, or to give it support. All skeletal structures, many protective structures, etc., have to be included here. While they add much to the gross bulk of the body, they add very little to the total metabolic changes in the body.

The great heat loss of the body takes place through the skin. In comparing the heat losses of similar animals, it is found that these losses are, in general, proportional to the area of the body surfaces of the animals concerned. In general, the body surfaces can be calculated from the formula used by Meeh,

$$S = K\sqrt[3]{W^2}$$

in which S = area, in square centimeters.

K = constant.

W = weight, in kilograms.

In the mammals the following constants have been determined:

Man	12.3
Dog	11.2–10.3
Rabbit	12.9–12.0
Rabbit-ears	10.8
Calf	10.5
Sheep	12.1
Cat	9.9
Pig	8.7
Guineapig	8.5
Fowl	10.4
Rat	9.1
White mouse	11.4

Du Bois and others have found that the formula $S = K\sqrt[3]{W^2}$ does not apply satisfactorily for determining surface area in many human beings, there being in some cases a deviation of as much as 16 per cent from the true value. There has been substituted, therefore, for Meeh's formula the following:

$$A = W^{½} \times H^{½} \times 167.2,$$

in which A = area, in square meters.

W = weight, in kilograms.
H = height, in centimeters.

This gives an average error of 2.2 per cent, which may be reduced to 1.7 by a slight modification, so as to read

$$A = W^{0.425} \times H^{0.725} \times 71.84.$$

This is the formula now used in most basal metabolism determinations upon human beings.

Meeh found 34.7 calories per square meter per hour as the measure of basal metabolism, in people of normal shape. More recent investigators show slight variations from the figure, depending upon age and size.

Relation of Cell Surface to Body Surface.—Various attempts have been made to correlate the total areas of the cells composing the organism with the total exposed body surface, in order to explain various facts of heat production and heat loss. Rubner estimated that each kilogram of body weight of a man possesses 150.2 square meters of cell surface, and that there is produced each day 0.2 calorie per square meter. A young mouse produces 2.2 calories per day per square meter of cell surface eleven times as much. There is, therefore, in these forms no possibility of making any such correlation.

Factors Regulating Heat Loss.—Among the factors which regulate the loss of heat from the body are the following physical factors: (1) regulation of blood flow through skin and its cooling through the action of the vasomotor nerves; (2) regulation of sweat production through sweat-secreting nerves; (3) respiratory center; and (4) water content of blood.

Much heat is lost through conduction and radiation from the skin. Vierordt estimates that 73 per cent of the heat of the human body is lost in this way. The surface of many lower animals is protected against excessive heat loss by fur or feathers. The human being has become so accustomed to the use of artificial means of preventing temperature changes that the natural forms of heat insulation have largely been lost. Under ordinary conditions the atmosphere surrounding the body has a lower temperature than the body itself. The difference between the temperature of the body and that of the surrounding air is also a factor in heat loss. The greater the difference in temperature the more rapidly

will heat be lost from the body. If there is a somewhat stagnant layer of air next to the body it will act as an insulator and prevent too rapid change of temperature. Such an air layer may be maintained by clothing.

As the warm blood circulates through the vessels of the skin, it comes into close relation to the surface layer of the skin, which is cooled by the external atmosphere. The rate at which the blood is passed through the superficial capillaries, and therefore the rate at which heat can be lost from the circulating blood, is regulated by the vasomotor nerves, which arise in the medulla oblongata.

The rate of blood flow through the vessels of the skin is controlled through an automatic reflex through the vasomotor nerves and the nerves controlling the sweat glands. Exposure of the skin to a warm temperature brings about a relaxation of the vasoconstrictor fibers of the skin arteries, and a greater quantity of blood flowing near the surface. Also exposure of the skin to warmth leads to a reflex stimulation of the sweat glands, and the pouring out of more water upon the body surface. The evaporation of this liquid from the surface requires heat, about 0.57 calorie of heat being lost for each gram of water so vaporized. Animals which have few sweat glands or in which the surface of the body is protected by hair are not able to secure the same cooling effects as do those whose skins are directly exposed to the air. In such forms, cooling is brought about by more rapid respiratory movements, during which water is evaporated more rapidly from respiratory surfaces.

The reflex effect of cold upon the animal body is opposite to that of warmth. Stimulation by cold leads to a constriction of the superficial blood vessels, a reduction in the amount of blood reaching the surface, and a reduction in heat loss. At the same time less perspiration is secreted upon the surface of the body, and therefore less heat loss through evaporation.

Physiological Oxidations.—The oxidations of the living animal body have been likened to the oxidations of organic material at high temperatures. Lavoisier recognized that in each case there is a combination of oxygen with the organic substance. He believed that in each case the combination is a direct combination of molecules of oxygen with the molecules of the substance oxidized, and that oxidations using equal amounts of oxygen should involve equal amounts of heat produced. An English investigator, Crawford, showed in 1777 that combustion of carbon, of wax, and the oxidation of tissue by a living guineapig gave off approximately equal amounts of heat for equal amounts of oxygen used.

For each 100 ounces of oxygen consumed by wax, the water of his calorimeter was raised 2.1°F. For each 100 ounces of oxygen consumed by carbon, the water of his calorimeter was raised 1.95°F. For each 100 ounces of oxygen consumed by a guineapig, the water of his calorimeter was raised 1.73°F.

The important fact here is that the rate at which the oxidation occurs is of no consequence so far as the total heat production of the reaction is concerned. At ordinary temperatures direct oxidations, *i.e.*, direct combination between a substance and oxygen, go on very slowly. In the animal body, oxidations take place much more rapidly than they would in the open air, but at a much lower temperature than is common for direct oxidations. Also the end products are the same whether the oxidation takes place in the living animal body or outside. The problem has been to explain how oxidation processes can take place so readily at low temperatures. Various suggestions have been made to explain the fact. Of these the one which has commanded most respect is the suggestion of Traube that there are in the animal body substances which, combined with oxygen, have the form of organic peroxides, and are then capable of bringing about the oxidation of organic material. There is no indication that the rate of oxidation is at all dependent upon the concentration of oxygen, provided a certain minimum amount be present, nor is there any indication that the oxidation affects the living substance itself. The oxidative processes are carried on by the aid of certain enzymes, oxidasis.

CHAPTER XXV

ENDOCRINE GLANDS AND THEIR WORK

There has been for some years a growing conviction on the part of biologists in general, and medical men in particular, that some of the apparently unimportant glandular structures of the animal body are of immense importance so far as the development, growth, and proper functioning of the animal body are concerned, and the suggestion now comes to mind that perhaps even more than body form is to be accounted for through the consideration of the effects of the secretions of these glands of internal secretion.

By *glands of internal secretion* are meant such structures in the animal body as evidently produce some substance which is of use in the work of the organism, but which is not carried off from the gland through any well-recognized tube or duct. Whatever secretion is formed is passed from the cells of the gland directly into the blood stream as the fluid circulates among the rather loosely aggregated cells.

Up to the present time it is not known how many structures in the body may be organs of secretion. Among those which are definitely known to be such are the thyroid glands, the parathyroids, the thymus, the pituitary, the pineal, the ovary, the testes, the placenta, the adrenal, and the pancreas. The mucosa of the stomach and the small intestine, possibly the mucosa of the vermiform appendix, along with muscle, are to be included in the list of structures producing chemical substances capable of regulating some of the biochemical processes of the living organism. It is, of course, in such a place as this, impossible to review all the evidence as to the nature of these structures. While the presence in the higher organisms of gland-like organs without proper ducts has been known for thousands of years, it was not until the eighteenth century that Theophile De Bordeu, a physician of Paris, made the first suggestion indicative of a budding understanding of the real function of some of these organs. Many of the earlier investigators in zoölogy failed to find that the glands of internal secretion were of any importance. Even Johannes Müller stated: "The ductless glands are alike in one particular—they either produce a change in the blood which circulates through them, or the lymph which they elaborate plays a special rôle in the formation of blood or chyle." And Henle wrote: "They have no influence on animal life: they may be extirpated or they degenerate without sensation or motion suffering in the least." A few years later (1849), A. A. Berthold of

Göttingen began an experimental study of the effects of the operative removal from fowls of the testes with a view to determining whether there be such a thing as an internal secretion as distinct from the external secretion of the gland. The experiments were well conceived and wholly successful in that they showed that a gland might at the same time function as a gland of external and of internal secretion, that these two functions are independent of each other, and presumably produced by different cells in the organ.

Within a few years after Berthold's work three other investigators, Claude Bernard, Addison, and Brown-Sequard, did experimental work which has had an important place in the development of our ideas of internal secretion. Claude Bernard's contribution had to do with an explanation of the disease diabetes. He made determinations of the amount of sugar present in the blood in different regions of the body, found that, in general, the arteries contained a higher percentage of sugar than the veins, and further that sugar was added to the blood during its circulation through the liver. He then found in the liver a store of glycogen, an animal starch, which evidently acted as the source of supply of the increased sugar in the blood. All this pointed directly to the idea that a given gland may have more than one function. Bernard seized upon this fact to emphasize the notion of internal secretions and their importance to the organism. It is not now customary to think of glycogen as an internal secretion but as a storage supply, but the work which he did was an undoubted stimulus to the investigation of those glands which have come to be regarded as the laboratories in which are formed numerous substances acting as starters or inhibitors of various physiological processes.

Almost coincident with the work of Claude Bernard was that of Thomas Addison (1855–1856), who published a monograph upon the constitutional and local effects of the suprarenal bodies. The condition then described, tuberculosis of the adrenal bodies, has come to be commonly known as Addison's disease. Addison described the effects of the disease and ascribed those effects to the lack in the body of something normally produced by the adrenal glands. He did not, however, go on to an experimental test of his theories. This was reserved for Brown-Sequard.

Brown-Sequard was the son of an American sea captain, a Mr. Brown, and a French woman, Madame Sequard. He removed one or both adrenal glands in several species of animals and found that when the glands were wholly removed there resulted a period of great weakness followed shortly by death. It was also found possible to prevent death for a long time if blood from a normal individual were transfused into the operated animal.

Nature of Endocrine Secretions.—Chemical substances which are introduced into the body are, in general, of one of two classes: toxins or drugs. The effect of toxins upon the living organism when they are in

one way or another introduced into the living tissues is shown by certain characteristic reactions, which are specific for one or another of the tissues of the body. They also tend, when introduced into the body, to give rise to the formation of certain substances known as antitoxins, the general effect of which is to neutralize the effect of the toxic substance. Another class of chemical substances, when introduced into the living organism, also gives rise to more or less specific reactions on the part of the different specialized tissues. These substances differ from the toxins in that they do not give rise to the formation of antibodies. Such substances are the drugs.

It has in recent years come to be recognized that each organism has for itself the power to produce in its tissues certain powerful substances which, taken into the blood and circulated freely through the organism, have the power to rouse to action or to modify the action of some particular organ or organs. These are the hormones.

These bodies have in common certain properties by which they may be recognized:

1. They are not rendered inactive by boiling; in this respect they differ from the enzymes. It was for a time thought that some of these substances might be enzymatic in nature. This idea appears for the most part now to have been given up. They are much simpler chemical bodies than the enzymes.

2. They are dialyzable. This indicates beyond any serious question that they are of relatively simple chemical constitution. At any rate, their molecules are small enough so that they are able readily to pass through the membranes of the cells of the body—a thing which molecules as large as enzymes are not able readily to do.

3. One at least (adrenalin) of these substances has already been synthesized in the laboratory. Its chemical formula is $C_6H_3(OH)_2.CHOH.CH_2.NH.CH_3$. The chemical composition of some others is known, and there appears little reason to doubt that others will be isolated, their molecular composition determined, and their synthesis effected.

4. Many of these substances are almost instantaneous in their action. In this they act in a way similar to many of the drugs. Such rapid action could hardly be imagined to follow upon the appearance in the blood or lymph of substances of very high molecular weight, and consequent low diffusion rate.

5. Some of these substances act in the body very slowly. Or perhaps it may better be stated that they act in very low concentration over very extended periods of time. These are the substances which exert their influences upon the growth and nutrition of the body.

6. None of these substances causes the formation within the body of antibodies when introduced from a foreign source.

7. The action of the hormones is similar in all animals.

It has become common to speak of the messenger bodies as being of two distinct classes in so far as their effects upon physiological processes are concerned; those which are excitant are termed *hormones*, and those which have a depressant or inhibitive action are termed *chalones*.

The Rôle of the Endocrines.—The problem before the physiologist, so far as the endocrine glands are concerned, is to make an analysis of the effects of the various glandular extracts upon the body, to decide as to what is normal and what is abnormal, and to relate to the activities of the glands the different skeletal types, peculiarities of stature and growth, shape and size of hands, fingers and toes, facial contours, teeth, skin, hair, eyes, muscles, etc. The whole problem of sex, its cause, the development of secondary sex characters, the rôles of the various glands in the phenomena of puberty, the functioning of the sex glands and of the whole reproductive apparatus are all bound up in a consideration of the ductless glands and their secretions.

The ductless glands and the significance of their secretions have challanged the interest and resourcefulness of many investigators. The field of investigation is one in which the danger of inaccurately controlled experimentation is unusually great. The experimental findings have been in many cases somewhat spectacular. The problems under consideration involve the interactions of a considerable number of unknown quantities—more than the ordinary student is accustomed to deal with. Problems involving one or two or even three unknown variables may be handled by the experimenter with some hope that a satisfactory solution may be obtained. Experimentation upon the ductless glands of one of the higher animals involves an unknown number of variables, and therefore calls for the most exacting and critical planning and procedure.

A great variety of general methods of work has been developed by different investigators in their attempts to check their own and others' mistakes and to eliminate all possible sources of error. Among the methods employed, in addition to the observations which have been made upon pathological cases, the following may be mentioned: (1) gradual extirpation of the gland; (2) feeding of glands, of extracts of glands, and more recently of their active principles, or of synthetic substances supposed to have the same composition as the active principles of the glands; (3) injection of glandular extracts, either subcutaneously or intravenously; and (4) vividiffusion (Abel). The last consists of running blood from an artery into an apparatus and back into a vein (by natural flow) and allowing diffusion of blood hormones from the blood to the outside. Tests are then made for these hormones.

The Thymus Gland.—The thymus gland is located in the upper chest cavity along the trachea, and overlaps the greater blood vessels as they leave the heart. In the higher mammals the gland arises from the epithelium of the third pair of branchial pouches of the embryo. In

some of the lower forms, as, for example, the fishes, it arises as a paired organ from the dorsal parts of all five of the branchial clefts; in Lepidosiren, from all except the first; in the Urodeles, from the third, fourth, and fifth; and in the Anura, from the second only. In all the fishes it is located dorsal to the gill arches on each side; in the amphibians it is close to the articulation of the mandible; in the reptiles it is found close to the carotid artery, but in young crocodiles and in birds it extends along the neck, lying close to the side of the œsophagus.

The thymus is composed of two portions, the corticle and the medullary, arranged in a lobular manner. The cortex is composed of lymphoid tissue and is very similar to a lymphatic gland in appearance. The medullary portion of the gland contains less of the lymphoid tissue, and is characterized by the presence of nests of epithelial cells, termed the *concentric corpuscles* of Hassall. The gland is found in all the vertebrates from Petromyzon up. So far as is known, no similar gland has been described as occurring in any of the invertebrates.

The period of activity of the gland is during very early life. In the human, the gland is functional at birth, increases in size somewhat during the first 2 years, and then normally begins its decline, which ordinarily becomes more rapid and is completed about the time of puberty. In individuals in whom the gland persists in an active condition beyond the normal time of puberty, there is a lengthened childhood.

Whether the gland should be considered one of the glands of internal secretion has been a matter of doubt in the minds of a good many investigators. No one has yet succeeded in isolating any substance which could be considered as the secretion of the gland. So, while absolute proof is lacking, there is considerable good evidence that the gland is one of the internally secreting glands.

The general action of the gland appears to be as an inhibitor of the other glands of secretion. It has the effect of keeping children childish, and in some cases of making children out of grown-ups. While present actively in the body it retards differentiation. Even thymus glands fed to growing animals similarly retard their development. The rise and the growth of the gland coincide with the period of childhood; the decline of the activity of the gland marks the period of puberty and the rise in activity of the sex glands. It acts therefore as a brake upon the other glands of the body which normally lead to development and differentiation.

When the gland is too active, the individual presents the condition known as the *status lymphaticus*. This condition is found in children and in some adults, and is characterized by the enlargement of the lymphoid structures throughout the body, and more particularly by the enlargement of the thymus gland. The condition is often a serious one because its existence may not be recognized until after some slight injury, shock,

exertion, or the administration of an anæsthetic have brought on fatal results.

An examination of the gland will in some cases give evidence as to the previous condition of nutrition of the body. A period of underfeeding for a short as time as 4 weeks will reduce the gland to one-thirtieth its normal size, or treatment by means of x-rays may be of real benefit.

Thyroid Gland.—The thyroid gland is a deep-red glandular mass consisting of two lobes lying one on either side of the trachea and the lower portion of the larynx. The two lobes are frequently connected by an isthmus. The essential portion of the gland is composed of numerous closed tubular alveoli or vesicles, lined with a layer of columnar epithelial cells which are not supported upon a basement membrane. These vesicles contain a colloid or jelly-like material. They are supported by fibrous septæ which grow inward from the gland capsule. The gland contains also many lymphatic vessels which have been shown to contain the same colloid material as the vesicles. There is also an abundant vascular supply.

The gland has its origin from the endoderm of the pharyngeal floor between the first and second branchial pouches and is found in all the vertebrates. The first appearance of the gland appears to be in the endostyle of the tunicates. The thyroid is in part, at least, under nervous control, being supplied with fibers from the sympathetic nerve.

The thyroid gland is effective during life and furnishes for the organism a definite substance, thyroxin, of the formula $C_{11}H_{10}O_3NI_3$ and with a molecule very similar to that of tryptophane. The substance occurs only in small amounts in the gland, but minimal quantities appear absolutely essential to the well-being of the individual. Some idea of the small amount found in a fresh gland may be derived from the fact that from $3\frac{1}{2}$ tons of fresh thyroid glands only 36 grams of crystalline throxin were obtained.

The general effect of the gland is to control the rate of energy transformations in the body. One milligram of the thyroxin fed to a person of 150 pounds weight suffices to increase the rate of metabolism 2 per cent for one day, and as the secretion of the gland controls the metabolic rate it will appear that it must also be concerned with the differentiation of tissues and the regulation of the shape and finish of organs. It seems the business of the gland to maintain in the blood a certain definite though small concentration of an iodine compound. In doing so it may, as has been suggested, maintain in the blood for the use of the body the iodine content of the sea, and may thus have made possible the evolution of the land animal.

In addition to the general work of regulating the rate of metabolism of the body there are other functions in which the secretion appears of importance. The gland appears to have a part in the neutralization of

bacterial and other poisons in the body and has a direct bearing upon the condition of the inner and outer coverings of the body, the skin, mucous membranes, etc., and upon the irritability and preparedness for response by nerves.

Interest in the thyroid gland and its rôle in the organism was very much stimulated by the investigations of Gudernatsch. This author found, and his work has been confirmed in its essential features by many other observers, that the metamorphosis of frog tadpoles and of salamander larvæ into the adult forms takes place at a date much earlier than usual if they are fed upon a diet which contains thyroid gland. The tadpoles become highly differentiated within a very short time. They develop legs and absorb their tails while they are still very young and very small, and they fail, as a rule, to develop to normal size.

"Without thyroid, no thought, no growth, no distinctive humanity or animality is possible." "Without thyroid there can be no complexity of thought, no learning, no education, no habit formation, no responsive energy for situations, as well as no physical unfolding of faculty and function, and no reproduction of kind, with no sign of adolescence at the expected age and no exhibition of sex tendencies thereafter."

The Adrenal Glands.—The adrenal glands are structures which are closely associated with the kidneys. Each is composed of two portions, a cortical portion which has its origin in the embryo from the cœlomic epithelium covering the mesoderm of the upper portion of the Wolffian body and corresponds to the mesoderm of the mesonephridial tubules, or, as Kanosik contends, arises from the germinal epithelium covering the upper portion of the genital ridge, and a medullary portion which has its origin from certain neuroblasts of the sympathetic system. Dr. Minot contends that these original medullary cells are later replaced by cells which grow into the medullary portion from the cortical layers.

Adrenal glands are to be found in nearly all of the vertebrates, though they are not found as distinct structures at any rate in the amphibians and Dipnoi. The presence of distinct adrenals is disputed in the Cyclostomata. In the elasmobranchs and the Holocephali there appears a separation between the medullary and corticle portions, the medullary portion being represented by a series of organs located in close association with the intercostal arteries, while the corticle portions may be either single or paired, and, being placed between the kidneys, are spoken of as interrenals. Among the amphibia, the glands are partially embedded in the substance of the kidney. In the reptiles and birds they lie rather close to the testes or ovary, while in the lower mammals they are not so closely connected with the kidneys as they are in man.

Among the invertebrates, the substance of the adrenal glands appears to be represented by cells of the nervous system which present the typical

chemical reactions of adrenal medullary cells to reagents containing chromic acid. Such cells are found among the annelids. Cells giving similar reactions are also found in the mantles of certain of the gastropods. Evidence is therefore not lacking that the presence of cells able to produce adrenal secretion occurs very generally in the animal kingdom.

The period of activity of these glands appears to be during the whole of life. As will appear later in the discussion, it is probably true that these glands do not operate at a constant rate, but that they store up secretion against a time of stress. The substance produced by the medullary portion of the gland is commonly known as adrenalin. It has the chemical composition $C_6H_3(OH)_2CHOH.CH_2.NH.CH_3$. This is one of the few hormones which have up to date been isolated in pure form. It has also been synthesized in the laboratory. It is normally present in the blood in about the ratio of 1:20,000,000.

It may be stated that the adrenal gland is a gland of masculinity and then an emergency structure acting as a gland of combat and fight. The most evident function of the secretion of the gland is in relation to blood pressure. A certain amount of the secretion, adrenalin, is necessary in the blood to maintain the tone of the smooth muscle fibers of the arterioles. As it controls blood pressure, it controls in a measure the distribution of blood throughout the body, an increase in the amount or concentration of adrenalin in the blood tending to send the blood from the viscera, where it is likely to stagnate during periods of lowered pressure, out to the muscles and brain where there may be special need for additional blood supplies. At the same time there will be thrown into the blood a greater number of red corpuscles, the heart rate will be accelerated, and the body temperature gradually raised. An excess of adrenalin in the blood leads to a high blood pressure, which may be so high or continued for so long a time as to become dangerous. Associated with the increase of the blood flow to the brain and muscles under the action of the substance there is an increased capacity for work. Failure of the gland to operate in a normal way leads to muscular fatigue, sensitiveness to cold, cold hands and feet, mental indecision (cold feet?), shock, collapse, and heart failure. Beard's neurosthenia has been accounted for as due to the failure of this gland.

Adrenalin in Annelids.—J. F. Gaskell calls attention to the intimate relation existing between the sympathetic nervous system and the adrenalin-secreting cells of the medulla of the adrenal gland. The cells whose business it is to secrete adrenalin may always be recognized by their chrome-staining reactions. In the higher mammals the cells secreting adrenalin are concentrated in the adrenal glands, while in the lower vertebrates adrenalin-secreting cells are to be found widely distributed, especially in all the sympathetic ganglia, and as in the lower vertebrates the sympathetic nervous system becomes less and less well defined there

is a more diffuse distribution of chrome-staining cells, sometimes segmentally arranged, throughout the animal body.

The so-called chromaffin cells have been very generally acknowledged by histologists to be the cells among the vertebrates which secrete adrenalin. Various investigators have attempted to find among the invertebrates cells of similar staining reactions, which may therefore be assumed to be adrenalin-secreting cells. Poll and Sommer found such cells in the leech Hirudo. Similar cells have been found also among the molluscs. Gaskell examined seventeen different Polychæte annelids and found chromaffin cells in two only, *Aphrodite culeata* and *Eunice gigantea*. *Lumbricus herculeus* also possesses them. Gaskell finds that the chromaffin cells are large ganglion cells, known in some forms as the giant cells. He raises question as to whether they innervate some special structures which are susceptible to the action of adrenalin. In answer to this query he finds that the chromaffin cells are found only in those forms in which there is developed a definite vascular system with muscular walls. The forms among the worms which possess chromaffin cells also possess definite muscular-vascular systems.

Pituitary Gland.—The pituitary body is a small glandular structure located at the base of the brain, back of the root of the nose. Its size normally is about that of a pea. The gland lies in a bony box, the sella-turcica, which often serves to confine it within such limited bounds as to interfere with its proper working.

The gland has a double origin, the anterior portion of the gland arising in the embryo from an ingrowth of cells of the ectoderm of the mouth area and from almost the same area as the thyroid. The posterior portion of the gland arises as an evagination from the floor of the nervous system almost immediately above the mouth. In some forms the parts of the gland arising from these two sources become very intimately related to each other, in other forms a separation of the anterior from the posterior portion may be accomplished without great difficulty, after the gland is once uncovered.

The pituitary is a gland which is to be found in all the vertebrates from myxine to man. It is functional normally throughout life, but is more active after the second year than before.

Two hormones have been isolated from this gland, both of which diffuse directly into the fluid bathing the nervous system. From the anterior portions of the gland there has been isolated a substance known as tethelin, and from the posterior portion a substance known as pituitrin. Acting along with the secretion of the thyroid gland these substances exert a profound influence over the growth and differentiation of the body. A large part of the effect of the anterior part of the gland is exerted upon the growth of bone and the connective tissues and muscle. An insufficiency of the secretion tethelin leads to dwarfism; muscular

fatigue; plump, tapering, fleshy hand, feet, toes, and fingers; full moon face with receding chin; flat nose. An increased secretion of tethelin appears to be responsible for gigantism, acromegaly associated with headache, muscular weakness, loss of sex activity, atrophy of sex glands, and an increased activity of the thyroid. Oversecretion also leads to the development of large, bony hands and feet, a long, narrow face with prominent bony parts, square jaws, emphasized eyebrows, cheekbones buried under a thick layer of fat; high, thin nose; large, irregular, square teeth; middle incisors especially prominent and developed in proportion to pituitary predominance.

This gland is also the gland of intellectuality. It seems to be tonic for the frontal lobes and in its activities lead to curiosity and to accuracy in judgment. It sensitizes the cells of the brain for mental growth, and is associated with philosophic, scientific, and intellectual development. It appears necessary for the preservation of memories. A deficient anterior pituitary secretion seems associated with the moron condition.

What is commonly spoken of as constructive imagination is very likely the expression of a certain balance in the activity of the two parts of the pituitary gland.

The posterior portion of the gland through the action of its hormone, pituitrin, controls the tone of the muscles of the blood vessels, bladder, and uterus, raises blood pressure, increases the flow of urine and of milk, and serves to regulate hibernation and probably sleep. Pressure or injury of this portion of the gland, resulting in diminished secretion or faulty secretion, leads to what is termed hypophysical adiposity. This is a general deposition of fat in all parts of the body.

In addition to these effects upon the body there are certain other more subtle effects to be noted. The secretion acts upon the basal ganglia that are the centers for emotional control, and so has to do with the regulation of the social and creative instincts, tender-heartedness, sympathy, and a religious feeling. When acting in excess, it leads to phobias, fears, anxiety neuroses, suspicions, hallucinations, delusions, and nervousness.

Parathyroid Glands.—The parathyroids are four small glands closely associated with the thyroid. They are developed from the pharangeal epithelium of the third and fourth visceral pouches. In the adult, two of them and sometimes all four may be embedded in the substance of the thyroid. It is due to this that much of the earlier work upon the thyroid gland was so unsatisfactory.

Histologically, the glands consist of solid masses of epithelioid cells, supplied with a rich vascular network, with small masses of what appears to be thymic tissue in the peripheral layer. These glands are found in the amphibia and in all groups higher. They persist during the lifetime of the individual.

No hormone has been isolated from these structures, but the effects of the removal of the glands from the body are so serious that it must be conceded that the glands are of the utmost importance. Removal of all the parathyroids simultaneously or at intervals results fatally within a few days. The symptoms exhibited are of a nervous nature. Excitability is greatly increased. The threshold of stimulation is greatly lowered, so that there is an irritability from 500 to 1000 per cent above the normal. All the reflexes are exalted. Fibrillar contractions are seen in the skeletal muscles of most animals; in man, tonic contractions occur. There are also cramp-like and clonic contractions leading into a condition of tetany in some of the lower animals. The pulse is quickened and the heart beat exaggerated. The salivary glands excrete excessively. The temperature of the body may be raised. There is a wasting of the body and a distinct loss of calcium salts. The hair falls out, bones soften, nails become brittle, and, in a young individual, teeth fail to develop. The whole list seems to indicate that the gland is responsible for the regulation of the calcium metabolism of the body and, therefore, of irritability of the cells of the body.

The Pancreas.—The pancreas is usually considered simply a gland for the production concerned with the intestinal digestion of food matters. Associated with the alveoli of the pancreas, lying bedded between them, arising from the same general source, the endoderm of the midgut, are small groups of cells known as the *Isles of Langerhans*. These groups of cells produce a substance known as insulin which is diffused directly into the blood stream and which serves, in cooperation with the secretion of the adrenal gland, to control the metabolism of sugar in the body. An insufficiency of insulin leads to that condition spoken of as diabetes.

The Pineal Gland.—The pineal gland is a small body projecting from the roof of the third ventricle of the brain, and above the pituitary. It arises as an evagination from the third ventricle. There are present at least two sorts of cells: nerve cells containing pigment and large glandular secreting cells, and associated with these are sandy grains of calcium salt. The gland is found in nearly all of the vertebrates, and in some of the lower forms has been interpreted as the remains of a third, median eye. Certain it is that the gland has attached to it a structure which has the appearance of retina and lens. In none of the higher forms is there indication that the gland has any visual function. On the contrary, there seems now very good evidence that the principal function of the gland is concerned with the secretion of some hormonic substance. What this substance may be is not known, for it has not yet been possible to isolate from the gland any substance giving rise to the conditions which are coming to be associated with the activity of the gland.

The gland is most active during childhood. It probably substitutes for the thymus as a general inhibitor of other endocrine glands after

the second year of life, and especially of the sex glands, and those having to do with the appearance of the secondary sex characters. Delayed involution of the gland leads to obesity. The gland must atrophy or retire from active service before the normal hypertrophy of the sex glands can start.

There seems also some connection between the gland and muscular system of the body. In cases of progressive muscular dystrophy there has been found a calcification, with accompanying inactivity of the gland. In precocious involution or destruction of the gland, hair appears upon the face and precocious puberty takes place in children of 6 years or less.

CHAPTER XXVI

PHYSIOLOGY OF MOVEMENT

There is no single property of living matter so striking as that of movement. As there is much variety in the structure of cells adapted for movement, so there exist gradations in the degree and effectiveness of movements. One may even go so far as to make a classification of movements based upon the characters of the cells making them. On this basis, movements are streaming, amœboid, ciliary (cirrous and membranelle), or contractile.

Streaming Movements.—Living protoplasm is not an inert substance, but one in which active streaming movements, at least at times, take place. This is particularly true in plant cells, as has been recognized by many botanists. The movements of the protoplasm in the cells of Chara or Nitella or of the stamen hairs of Tradescantia are classic examples. The phenomenon in animal cells is not usually so spectacular as in plant cells, and has therefore received less attention from zoölogical students. Streaming movements are common among the rhizopods and other protozoa, and have been noted in the colorless blood corpuscles of a wide range of animals, in some egg cells, in pigment cells, and in the intestinal epithelium cells of many of the metazoans, and may occur in other forms of cells as well. Beyond the fact that the streaming movement occurs, little is known of the phenomenon.

A number of theories have been proposed at various times to account for the observed facts. These may be classed under a few headings:

1. *Contractility Theories.*—In these, there has been an assumption of the existence of contractile elements such as fibrillæ in the cells, or there have been assumed waves of contraction passing along the walls of the cells. None of these theories has so far been substantiated by a sufficient accumulation of facts.

2. *Imbibition Theories.*—These assume the imbibition of water and its successive discharge by certain constituents of the cells. Waves of imbibition and extrusion passing progressively along the cells were thought to account for the streaming movements observed. No sign of water being extruded from such a cell has been seen. Further, the amount of liquid which must be thus imbibed and released in the course of a day in order to account for the movements is so large as to be beyond belief.

3. *Verworn's Oxidation Theory.*—This is based upon Verworn's postulate of the biogen molecule, a thing which probably does not exist.

4. *The Electrical Theories.* –These fall into three classes, the galvanic, the electromagnetic, and the electrochemical-surface-tension theory. Of all the theories suggested, that of Ewart is the best, but it falls far short of explaining the facts observed in many animal cells. Ewart has come to the conclusion that there are differences of electrical potential between the protoplasm-vacuole boundary and the protoplasm-cell wall, and that, as a consequence, electrical currents are passing between these two points, traversing the protoplasmic stream. If, now, it is assumed that the particles in the endoplasm, which are electrically polarized, have the surface tension of their corresponding ends decreased when electric currents traverse the endoplasmic stream, the particles, and of necessity the whole stream of endoplasm, would move in the direction of the lowered surface tension. Continuous chemical actions would be necessary to maintain the conditions outlined. This theory accords with the facts, as far as it goes, but it does not explain the streaming in threads across the vacuole in a plant cell, thus necessitating theories for the explanation of streaming within a single cell at the same time. Moreover, a central vacuole of cell sap seems always required to fulfil the conditions of this theory, and this, as is readily seen, makes it impossible to apply it to streaming in Amœbas, Myxomycetes, Foraminifera, and Ciliates.

The fundamental cause of streaming is, therefore, still to be discovered, for neither the theories of streaming as applied to the Amœba nor those described above which refer especially to plant cells are satisfactory. A significant point in these theories is that, with increasing information, they come more and more to demand a colloidal structure in the protoplasm. It is the surface energy in the interfaces of the colloidal system which comes to be regarded as the primary source of the energy of streaming. All attempts thus far to explain exactly just how this energy is utilized have been unsuccessful. Gaidukov's observation is of some interest in this connection. He found that the occasional stopping of streaming in cells of *Vallis neria* is accompanied by cessation of Brownian movement, which indicates a change from the sol to the gel state. This proves that the colloidal changes are possible in streaming protoplasm and that a general search for a general explanation of streaming along this line is proceeding in the right direction.

Amœboid Movement.—From the time when Berthold (1886) attempted to explain the movements of amœboid cells through the physical relations existing between the animal and its immediate environment, physiologists have been interested in some way to account for such movements upon purely physical principles. Since that time, many new facts in relation to protoplasmic movements have been discovered. As the variety of the forms of cells which exhibit movements is observed and the varieties of ways in which this power of movement is expressed are noted it is only natural to wonder whether all forms of the process, when reduced to the

lowest terms of analysis, have as their fundamental activities something in common. The movement of the muscle fiber is definitely a contractile process involving certain physicochemical surface phenomena occurring at interfaces within the fibers. There is reason to believe that the action of cilia and flagella is also due to some contractile process. Amœboid activity was at first explained as probably due to some changes in surface tension in the surface film of the animal, resulting from some factor in the environment.

A clear understanding of the process of amœboid movement demands a rather critical acquaintance with the normal structure and behavior of Amœba. The differentiation of the protoplasm of Amœba into ectoplasm and endoplasm is a fact familiar to all students of zoölogy. The term *ectoplasm* is sometimes (rather loosely) used to designate the clear, nongranular layer of protoplasm which thinly covers some of the more common Amœbæ and which is especially prominent in some of the small species where the larger part of the anterior end often consists of protoplasm quite free from granules. A better use of the word restricts its application to the more or less rigid and motionless layer of protoplasm on or near the outside of the Amœba. This resembles the gel state of a colloid. The term *hyoloplasm* would perhaps be a better term to apply to the whole outer clear layer. While the endoplasm of the Amœba is decidedly liquid in character, as may be seen from its streaming movements, the ectoplasm is much more rigid. If the upper surface of a pseudopod is examined carefully there will be seen minute particles or crystals embedded in the ectoplasm, which exhibit either very slight Brownian movements or none at all, indicating that the substance in which the crystals are embedded is a quite firm and rigid gel. There is no sharp line of demarcation between the more rigid and the more fluid parts of the protoplasm, the one grading gradually into the other.

The casual observer of an Amœba is hardly likely to appreciate the significance of these two sorts of protoplasm or the fact that there is in amœboid cells generally a practically continuous transformation of endoplasm into ectoplasm and of ectoplasm into endoplasm. The details of the process vary in different species, and in some forms are greatly complicated by the formation upon the surface of longitudinal ridges and of a coarse, somewhat spongy network within the pseudopodia.

In Amœba lauriata the body has the form of a nearly cylindrical tube. In the cytoplasm are embedded various minute nuclei and numerous small vacuoles each containing a crystal. Schaeffer says:

> In this Amœba the endoplasmic stream flows uniformly where it spreads out slightly so as to preserve the same general diameter of the Amœba, for it is characteristic of this Amœba that the anterior end is of about the same diameter as the posterior, when in clavate form. The ectoplasmic tube is built at the anterior end, and remains as constructed until it is drawn in at the posterior

to form endoplasm . . . The immobility of the ectoplasm is much more readily observed in *Amœba lauriata* than in perhaps any other species, a condition that is due chiefly to the large crystals whose displacement is the most convenient criterion of ectoplasmic mobility.

The ectoplasmic tube is not so thick as in *Amœba proteus*, though it appears to be more solid than in that species. It is thrown into folds at the posterior end as it is liquefied to form endoplasm which indicates a firm texture of the ectoplasm. As to the endoplasmic stream, it presents no visible characteristics which set it apart from the fluids of physics; it moves most rapidly in the middle, and gradually less actively as the ectoplasm is approached. There is no backward movement of the ectoplasm along the sides of the pseudopod at the anterior end—nothing approaching a *fountain current*, which indicates that the transformation of endoplasm into ectoplasm is rapid and complete. That is, all the endoplasm which reaches the anterior end is turned into ectoplasm. Typically, this would result, in an Amœba of average size, in a layer of ectoplasm of a thickness of about one-seventh of the diameter of the pseudopod (for the area of the cut ectoplasmic tube would equal the area of the endoplasmic stream). But because of friction against the sides of the ectoplasmic tube, there is a layer of endoplasm of appreciable thickness that is practically motionless. This layer of endoplasm, therefore, makes the diameter of the endoplasmic stream appear smaller than it actually is, and the ectoplasmic tube larger than it is. The actual thickness of the tube of ectoplasm is, as distinguished from the endoplasm, difficult to measure, but it seems to be about one-tenth the diameter of the pseudopod.

In other amœboid forms the details of the process of streaming and of the formation of ectoplasm vary within wide limits. In some forms the endoplasm is much more fluid than in the one described; in some forms the flowing, instead of being steady, takes upon an eruptive character; in some forms endoplasmic currents are turned backward from the anterior end just beneath the ectoplasmic layer for a shorter or longer distance; in some forms the endoplasm is less fluid and the ectoplasm relatively thicker than in the form described. Those interested in the descriptive details of these variations should consult Schaeffer's book, "Amœboid Movement." While at the present time there is no explanation of streaming movements, it is concluded that the surface film, that outer layer of ectoplasm in direct contact with the water, is essential for locomotion. The outer film is a true surface-tension film and has its region of highest tension at the point where new ectoplasm is being most rapidly formed.

Cilia.—In all groups of animals from the protozoa up there are found cells which bear, upon their free surfaces, hair-like projections (cilia and flagella) capable of making lashing movements. Investigation has shown that the cilia form only a part of a rather complicated mechanism, the ciliary apparatus (see Figs. 62 and 63), which consists of cilia, basal corpuscles, and rootlets. In certain protozoön forms, it has been further shown that the ciliary movements are under the control of an intracellu-

lar neuromotor apparatus. Especially is this true in such forms as have the cilia masses and are bound together by protoplasmic films into such structures as cirri and membranelles. It is not to be supposed that these mechanisms are equally well differentiated in all cells, nor in any individual cell at all times. In some cells the cilia are not permanent structures, and the cells at different periods in their life histories assume quite different functions and appearances. The ciliary apparatus is produced by the differentiation of the mitochondria of the cell, and are not, as has been stated by some authors, simply ectoplasmic modifications of the character of pseudopodia. The cilia, formed within the cell, are pushed

Fig. 62.

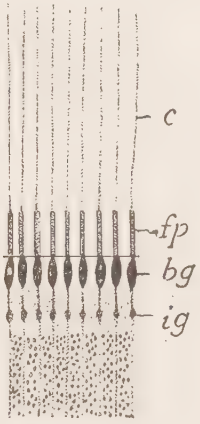


Fig. 63.

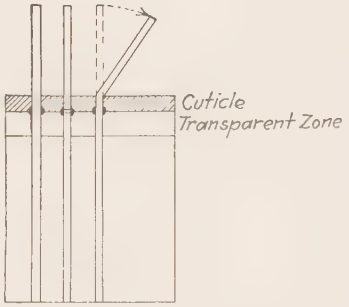


Fig. 64.

Fig. 62.—Ciliated cells from the alimentary tract of *Anodonta mutabilis*. *Co*, collar; *ba.g.*, basal granule; *i.g.*, inner granule; *n.m.*, neuromotor apparatus; *n.*, nucleus.

Fig. 63.—Outer end of ciliated epithelium cells—diagrammatic. *fp*, foot piece; *bg*, basal granule; *ig*, inner granule; *c*, cilia. (*After Joseph, from Schneider.*)

Fig. 64.—Diagram showing Saguchi's conception of the structure of a cilium.

outward from the endoplasm through the ectoplasm and cuticle. In some cells the cilia appear even retractile in character. The accompanying diagram (Fig. 64) taken from Saguchi's account of ciliary structure in the *Journal of Morphology* is an attempt to represent the facts as they were understood by him.

Some authors have held, upon rather doubtful evidence, that the cilia are made up of more than one kind of substance. Most authorities are agreed, however, that there is no structural differentiation in the cilium itself. The cilium, passing downward through the cuticle, the basal granule, and the rootlet anchored in the clear ectoplasmic layer, forms a continuous structure ending in the cytoplasmic strand or fiber which may or may not be in connection with an intracellular neuromotor

apparatus. In either case the cytoplasmic fibriles undoubtedly serve as a means of coordination between the cilia and the other portions of the cell. Possibly they may have both a conducting and a contracting rôle to play. As the cytoplasmic strands pass backward into the cell they frequently converge, forming a cone-like structure with its apex at one side of the nucleus. In the region of the apex the fibers become lost in the cytoplasm of the cell.

The cilium has no motive power of itself. The ciliary movement appears due to some activity of the basal granules. Saguchi's conception is that the basal end of the cilium and the distal end of the rootlet are connected either directly or by means of a joint, around which is the basal granule. The latter, like muscle substance, is endowed with contractility, by virtue of which the cilium is bent at that point; hence the rootlet acts as a support of the cilium, while the transparent zone holds the rootlet in its position; the restoration of the bent cilium may be affected by the elasticity of the cilium itself or by the antagonistic action of the contractile substance. The stimulus which causes the waving of the cilia may be conceived as being conducted directly from one basal granule or corpuscle to another or through the neuromotor apparatus.

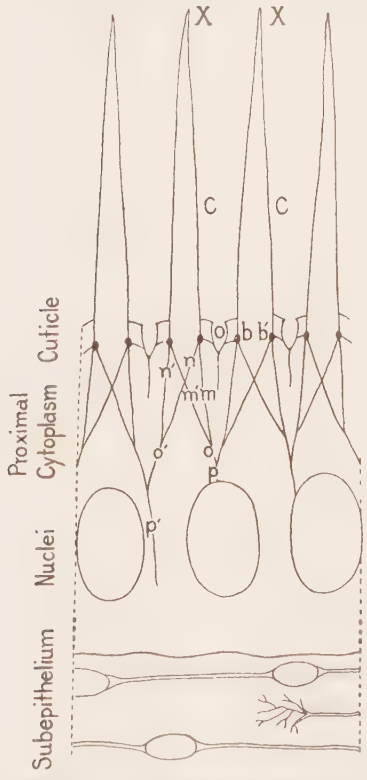


FIG. 65.—Diagram illustrating ciliary structure as demonstrated by Grave and Schmitt. For description see text.

Structural Basis of Coordinated Ciliary Action.—Grave and Schmitt have recently given an explanation of ciliary action which concerns not only the movement of the individual cilia but also the coordination of the activities of adjacent and neighboring cilia. They worked upon the laterofrontal ciliated epithelium of the gill of a species of Lampsilis. The cilia *C* (Figs. 65 and 66) in this type of epithelium are paired, and the members of pairs are fused at their tips *X*. The number of pairs of cilia is approximately the same as the number of the nuclei in the syncytial epithelium. A specialized cuticle is present at the external surface of the epithelium in which the cilia and their basal corpuscles *b* are implanted. In the proximal zone of the cytoplasm, between the cuticle and the row

of nuclei, a system of intracellular fibers or ciliary rootlets is chiefly distributed, although fibers belonging to the system may penetrate the cytoplasm between nuclei and perhaps even to the basement membrane and the subepithelial layer of bipolar cells. This system of intracellular fibers may be analyzed as follows: Each cilium spilts, just below its basal corpuscle, into two fibers m and n which diverge at angles varying from 20 to 30 degrees, one to the right and one to the left in the plane of the row of cilia. The right-hand branches m and m' originating from the members of a pair of cilia gradually converge and join to form a single fiber o, while the left-hand branches n and n' join to form the fiber o'. The fiber o and o' join with corresponding fibers derived from adjacent pairs of cilia to form the fibers p and p', which may end on the nuclear membrane or penetrate the cytoplasm between nuclei. The part of this system consisting of the fibers m, m', n, n', o, and o' forms a mechanism which is continuous throughout the extent of the epithelium and is postulated as that which provides a basis for the coordination of ciliary movement. The continuations of this system, through the fibers p and p', etc., into the deeper parts of the epithelium provide for the entry into the system of impulses, originating either in nuclei, the cytoplasm, or bipolar cells of the subepithelium, by which movements of the cilia may be adaptively regulated in conformity with the state or needs of the organism as a whole.

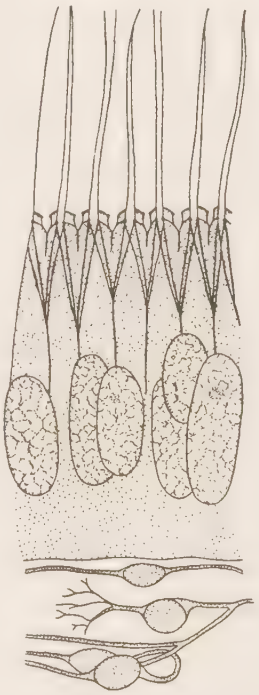


FIG. 66.—Grave and Schmitt sketch showing actual arrangement of ciliated cells. Note the neuromotor fibers extending from the region of the nuclei to the bases of the cilia, also the cells of the nervous plexus beneath the epithelium.

In addition to the mechanism for the coordination of ciliary movement just considered, another series of structures is present in each of the segments of the cuticle bounded by pairs of cilia, which apparently serves another purpose. One of these systems may be described as follows: A minute space O interrupts the continuity of the cuticle midway between pairs of cilia in the plane of the series of cilia. This space opens on the surface of the epithelium and, in section, appears to be formed between two fiber-like structures r and l which join the cuticular border above in two minute granules and meet at the lower border of the cuticle midway between the bases of the cilia of two pairs in a similar granule. This latter granule is the meeting place also for two comparatively short, robust fibers R and L which originate, one from the nearest basal corpuscle of the pair of cilia on the right of the space, the other from

the nearest basal corpuscle of the pair on the left, and it is the point of origin for a longer, unpaired, vertical fiber *S*, which penetrates the cytoplasm to a depth below the cuticle about twice the thickness of the cuticle. This series of cuticular structures requires further study. The fibers *R* and *L* may be interpreted to be a part of the mechanism for the coordination of ciliary movement. The spaces in the cuticle and their attendant fiber-like differentiations possibly represent a structural adaptation for taking up stresses and strains in the highly gelled cuticle incident to ciliary movement.

It may not be too much to predict that similar structural differentiations will be found in all ciliated epithelia.

Physiological evidence in favor of such a structural system as has been described was furnished by Kraft in 1890, and has been referred to by Parker in his "The Elementary Nervous System," pages 68 to 70. Parker thinks of the coordinating elements not as nervous, but as neuroid. The transmission of impulses is sluggish, is limited in extent and in direction, and really represents a primitive protoplasmic conductivity.

Variations in the Irritability of Ciliated Cells.—It has been known for a long time that certain cells, *e.g.*, muscle cells, vary from time to time in their degree of irritability. Such variations have also been noted in ciliated cells, as, for example, in the ciliated cells of the gills of the mussel Mytilus. After the gills have been excised for some hours from the body of the mollusc there occurs an interruption of the automatic activity of the ciliated cells which is associated with an extreme depression of irritability. This weariness does not seem a symptom of approaching destruction, nor is it due to the accumulation of waste products. It has been interpreted as being due to overexcitement. It was found that an acceleration of ciliary movement followed soon after by weariness could be caused by adding to the sea water a small amount of alkali. In both cases the sensitiveness of the ciliated epithelium to mechanical shocks was very much reduced, but after a few hours was fully restored, so that the slightest agitation of the water induced automatic movement. A decrease of irritability could be directly produced without previous excitement by placing the epithelium in hypotonic sea water or by adding a small amount of acid. In either case the automatic movement soon stopped. Rubenstein has called attention to the close connection between mechanical irritability and automatic movement, and accepts the hypothesis put forward by Yerkes that automotism is a function of high irritability. The stoppage of the movement of the cilia described above is accounted for as follows: In normal tissue the movement of the water acted upon by the cilium furnishes a stimulus for the following contraction. If the irritability of the cilium be lowered, this stimulus does not reach the limit necessary to produce a new contraction and the automatic movement ceases. There is, therefore, a definite threshold value of stimulation here as in muscle tissue.

Work Done by Ciliated Cells.—It is possible, by placing a ciliated epithelium upon an inclined surface of known gradient and allowing the cilia to carry a load of known surface and weight against gravity, to determine the amount of work done by them. The time required to carry a given weight over a measured course is subject to variation because of the drying of the membrane and because of variations in the irritability of the cells due to the stimulation of operation and variations in the chemical conditions surrounding the cells. Probably the best medium to use for moistening the membrane is serum from the animal from which the membrane was taken, though a saline solution containing the same salts as occur in the blood, and in approximately the same proportions, serves admirably.

By calculating the work done in terms of gram-millimeters per minute per square centimeter, it is possible to make just comparisons of the work done by different ciliated cells under different conditions. Maxwell (1905) found as the average results of a series of ten experiments that with different loads the curve of the amount of work done by the cilia rises rapidly with increasing load, maintains itself near the maximum through a considerable range of load, and then falls rapidly to the point where the load cannot be moved at all. The figures given in the following table hardly represent accurately all of the work done by the cilia, for it must be remembered that they exert their energy upon the liquid by which the cilia are surrounded and not directly upon the load carried.

TABLE 49

Relative weight load	1	4	7	10	13	16	19	22
Absolute weight load	0.415	1.66	2.905	4.15	5.395	6.64	2.885	9.13
Work done	2.333	6.792	9.424	10.17	10.01	9.18	5.85	0.0

Maxwell also noted, when testing the behavior of cilia while carrying weights, that the rate at which the weight was carried was decidedly slower in the earlier than in the later trials. He suggested that the cilia become more irritable to succeeding stimuli, and that the effect noted is comparable in principle to the *staircase effect* seen in the contraction of striated muscle. He also called attention to the fact that cilia, by moving heavy weights, become fatigued, and that when so fatigued a short period of rest frequently restores them to their original rate of activity.

In considering the effect of solutions, each containing a single salt, upon ciliary movement Maxwell found that sodium chloride is the one most favorable for preserving the cilia in such condition that they are able to do mechanical work. Arranged in the order of their favorable action upon the working power of cilia the salts (all chlorides) stand as follows: Na, Sr, K, Ca, NH_3, Mg, Ba.

If the salts are arranged according to their power to maintain ciliary activity they seem to stand as follows: Na, Sr, Ba, Mg, Li, Ca, K, NH_3.

Effects of Ions upon Ciliary Movement.—Recently, Gray has made an attempt to determine the external conditions necessary for the maintenance of ciliary action. For his study he chose the ciliated gill plates of *Mytilus edulis*. Very early in the work it became apparent that the concentration of the hydrogen ion in the solutions employed was a factor of supreme importance. Attempts were made to determine the effects of the different metallic ions present in the sea water, but with only moderate success. It was possible to determine that solutions containing only one of the metallic ions found in the sea water were exceedingly injurious; that solutions containing two of the ions were less injurious than solutions containing only one; that solutions containing three ions support the tissue in a comparatively healthy state for as much as 72 hours; and that when all four of the principal metallic ions, *viz.*, Na, Ca, Mg, and K, are present, the tissue remains healthy and normal as in sea water. Of these ions, the Na proved the most toxic. Gray was not able to show that there is here any definite antagonistic action between the calcium and the sodium ions, for example, there being rather wide limits as to the concentrations in which the various ions might be present in the solution and still allow ciliary action to continue.

As a contrast to this, however, it was observed that ciliary activity was to a very great degree dependent upon the concentration of the hydrogen and hydroxyl ions. When the pH of the solution falls below 5.5 to 6.0 the cilia are unable to lash. Also, if the pH of the medium rises to 9.0 or above the ciliated epithelium breaks up rapidly into its constituent cells, but the ciliary movement does not stop either in the isolated cells or in the cells which remain in situ. Such breaking up and disintegration of the epithelium take place in all artificial solutions which do not contain sodium, potassium, magnesium, and calcium. Similar observations have been made upon the behavior of the spermatozoa of various marine invertebrates. There is a very definite and narrow possible range of hydrogen ion concentrations of the medium in which swimming movements of the spermatozoa may take place and, as a corollary, in which the spermatozoa may be effective as fertilizing agents. Similar facts have been determined for the spermatozoa of some of the mammals. It appears that a rather fundamental condition is here being dealt with.

R. S. Lillie (1906) published an account of the effects of various pure sodium salts upon the ciliary action of Mytilus. He found that the ions could be arranged in the following order of toxicity:

$$Cl' < NO_3' < Br' < I' < SCN'$$

He also found that ions affect the absorption of water by the cells from their external medium, as follows:

$CH_3COO' < Cl' < NO_3' < ClO_3' < Br' < I' < SCN' < BrO_3' < OH'$

These results have formed the basis of various textbook statements to the effect that: (1) the above series represents the effects of anions on ciliary movement; and (2) the order in which anions affect ciliary movement is the reverse of that in which they affect muscular movement.

Gray has reinvestigated the effects of ions upon the gill cilia of Mytilus and comes to conclusions quite different from those given above.

> Whereas in a solution of a sodium salt containing K′, Ca″, and Mg″ the ciliated epithelium is remarkably indifferent to a variation in the nature of the anions present, yet in a solution of a sodium salt which does not contain other cations the tissue shows a very marked sensitivity to particular anions. In other words, so long as the normal equilibrium of Na′, K′, Ca″, and Mg″ is maintained in the solution it makes little difference whether the anion be the Cl′, NO_3', Br′, I′, Acet′, or SO_4'.

Gray found also that in solutions containing tartrates and citrates the bivalent metals are probably not present in the ionic state, and the cells behave as if these ions were absent.

There is also no justification for the statement that the order in which anions affect ciliary action is the reverse of the order in which they affect muscular movement. When the cells are treated with pure sodium salt solutions there is a destruction of the normal semipermeability of the cell membrane, and the cell colloids behave as an elastic gel in direct contact with the external medium. The activity of the sodium salts in thus affecting the permeability of the cell membrane can be inhibited by magnesium or by calcium. Gray expresses the opinion that in the normal sea water the stability of the membrane is very likely due to the presence of the magnesium rather than of the calcium.

Gray also found that in balanced solutions the monovalent cations have a direct effect upon the rate of ciliary movement. The rate of movement in solutions having the same hydrogen-ion concentration is slowest in lithium and fastest in potassium. The ions can be arranged in the following series:

$$Li' < Na' < NH_4' < K'$$

It does not seem necessary to maintain the customary semipermeability of the cell wall in order to maintain ciliary movement.

Effect of Hydrogen-ion Concentration.—Gray found that mineral acids enter the ciliated cells of Mytilus very slowly, while weaker acids, such as acetic or butyric, penetrate rapidly. Cells which have been stained with neutral red show no change in color when placed in Van't Hoff's solution made acid with HCl to a pH of 3.4, while when placed in a similar solution acidified with acetic or butyric acids the cells instantly change from brick-red to bright-red color. Similarly, ammonia quickly

enters the cells, while NaOH does not. These facts make it possible to determine whether the cessation of movement of the cilia in an acid medium is due to interference with the electrical properties of the cell surface or whether the affected elements lie within the cell itself. Gray found that:

> The weak acids which enter the cell are more efficient inhibitors of ciliary movement than are the strong acids which do not enter readily, and conversely the weak alkalies are much more efficient restorers than the strong alkalies.

The effect of the acid is to cause the cilia to come to rest in an acid solution by a gradual diminution in the rate of the whole beat without any reduction in its amplitude. Furthermore, the cilia always come to rest at the end of the effective stroke, that is, in that position in which the cilium possesses no potential energy.

Cohn showed that when the movements of spermatozoa are stopped in an acid medium there is no loss of energy, but there is a cessation in the conversion of chemical into potential energy. This transformation of energy can be reestablished by removing the acid from the environment. It thus appears that the stoppage of the cilia by the acid is due to the fact that there is no longer a conversion of chemical into potential energy within the cell.

Effect of Temperature upon Ciliary Action.—The fact that ciliary action increases with a rise in temperature has been known for a long time. Calliburces studied the rate of movement of the cilia of the frog's œsophagus in 1858. Since that time numerous investigations have been made along this line, the most recent of which is that by Gray.

Gray studied the cilia of the gill of Mytilus. He found that at from 0 to 32.5°C. there is a progressive increase of speed with normal amplitude of stroke with increasing temperature; that between 32.5 and 36° the beat becomes still more rapid, but that the amplitude of the beat becomes reduced, so that at the higher temperature it may be spoken of as a rapid flickering movement; from 37.5 to 38.5° the speed of the beat becomes reduced; at 40° the cilia become stationary in the relaxed position; at 45° they pass into the contracted position; and at 47° they become opaque and irreversibly injured. The direct effects of temperatures between 0 and 36° are completely and instantaneously reversible. Between 36 and 40° the effects are also reversible, but recovery is not instantaneous. The following table taken from Gray's paper (*Proc.* Roy. Soc. Ser. B., vol. 95) shows the relative rates of ciliary action at different temperatures and the temperature coefficients of the same.

TABLE 50

Temperature, degrees Centigrade	Speed in millimeters per second	Relative speed at 15° = 100	Temperature interval	Q_{10}
0	0.08	20	0–10	3.1
5	0.15	38	5–15	2.7
10	0.26	64	10–20	2.3
15	0.40	100	15–25	2.15
20	0.60	150	20–30	1.95
22.5	0.70	174	22.5–32.5	1.92
25	0.86	215		
30	1.17	294		
32.5	1.33	334		

Two suggestions have been made as to the cause of the depressant effects of high temperatures upon biological processes, namely, that: (1) at high temperatures the cells suffer from lack of oxygen; and (2) at high temperatures one or more endocellular enzymes cease to be stable and are gradually destroyed.

In an attempt to decide which of these possibilities is correct Gray made a study of the effects of oxygen upon ciliary action. He found that between 0 and 30° the oxygen consumption is almost exactly proportional to the mechanical activity, when the tissue is well aerated. At temperatures above 30° the initial oxygen consumption is not maintained.

Gray says:

> The whole series of events which accompany continuous activity is divisible into at least three phases: (1) a reaction which is sensitive to monovalent cations (particularly the hydrogen ion), any interference with which involves a change in the rate of beat, and leads ultimately to a change in the amount of oxygen absorbed. This reaction is, however, not directly affected by a lack of oxygen, and in the absence of oxygen will continue for a considerable period. From this reaction is probably derived some substance of an acid nature. (2) A mechanism which is brought into operation by the presence of an activating acid substance, and which is sensitive to marked changes in the amount of water present in the cell. Calcium is a necessary constituent of this mechanism. The events associated with this mechanism are independent of and have no influence upon the amount of oxygen absorbed. (3) A reaction of an oxidative nature which is necessary for prolonged ciliary activity. The oxygen appears to remove some factor which is the direct result of activity and which if allowed to accumulate will inhibit the activity of the whole ciliary apparatus.

Gray is of the opinion that the facts point to the conclusion that the point of origin of the energy expended by the ciliated cells of Mytilus lies in an intracellular protein. He also calls attention to the very close parallel between the ciliary mechanism and cardiac muscle as illustrated by the sinus region of the heart.

Effect of Osmotic Pressure.—The cilia of Mytilus are not very sensitive to slight changes in the osmotic pressure of the liquid bathing them, yet if the change exceeds a certain value the cilia are brought to a complete standstill. Upon bringing the cilia back into water of the nor-

Comparison of Properties of Ciliary and Muscular Contractions (*Gray*)

Reagent	Cardiac muscle	Cilia of Mytilus
Temperature	Q_{10} for frog's sinus for interval 1.2°–30.5° = 3.1–1.3. Oxygen consumption proportional to rate of beat.	Q_{10} for interval 0°–32.5° = 3.1–1.92. Oxygen consumption proportional to rate of beat. Phases of heat rigor parallel to those of the heart.
Oxygen	Oxygen not directly required by the contractile mechanism but is required for prolonged activity.	Oxygen is not directly required by the contractile mechanism, but is required for prolonged activity.
Cyanide	Reaction of the two types of tissue the same.	
pH	Sinus of frog active within limits pH 4 to 9.5. Optimum speed 8.1.	Active within limits pH 5 to 9.5. Optimum about 9.0.
	Acids reduce speed. Excess of alkali reduces rate of relaxation.	Acids reduce speed. Excess of alkali reduces speed of relaxation.
Monovalent cations	Li′ beat slower than normal.	Li′ beat slower than normal.
	K′ increase in concentration on sinus quickens beat markedly.	K′ increase in concentration causes quickening of lateral cilia.
	K′ in presence of excess of Ca″ causes tonic contraction.	K′ in excess causes tonic contraction of frontolateral cilia.
Calcium	Absence of Ca″ causes stoppage in relaxed position. Ca″ can be partially replaced by hydroxyl ions.	Absence of Ca″ causes stoppage in relaxed position at pH 7.0. Ca″ can be partially replaced by hydroxyl ions.
Anions	If cations are balanced, changes of anions have little influence.	If cations are balanced, changes of anions have little influence.
Non-electrolytes	Pure dextrose supports beat for a considerable period.	Will beat normally in dextrose for a considerable period.
Osmotic pressure	The amplitude but not rate is affected in hypertonic solutions.	Reduction of amplitude but not rate in hypertonic solutions.
Veratrin	Can replace potassium, but the two agents are antagonistic.	Can replace potassium but is antagonistic to it.

mal concentration the ciliary activity is resumed. In a solution which is not quite strong enough to cause complete stoppage, a reduced movement is maintained for a long time. In such a case the amplitude of the beat is much reduced while the rate is very little affected. The stoppage in the hypertonic solution is due to the fact that the loss of water from the cell interferes directly with the contractile mechanism and not with the periodical liberation of energy.

Reversal of Ciliary Action.—One of the most common phenomena associated with ciliary action among the protozoa is that of the reversal of the direction of the beat. Such reversals also occur among many of the lower invertebrates, but no case of reversal has as yet been reported among the vertebrates. The reasons why ciliary reversal should occur among animals in which cilia are the chief locomotor, food-gathering, and protective structures are readily apparent. The conditions which render ciliary movement possible and those which may serve to bring about a reversal of the stroke are still open to inquiry.

Whether the conditions effective in causing a reversal are always the same is not known. In *Metridium marginatum*, which was investigated by Parker (1905), it was shown that the normal, undisturbed, expanded animal has the cilia of the siphonoglyphes beating so as to carry water currents inward toward the gastric cavity, and that on the lips and on the tentacles the water currents are directed outwards. When the cells of the lips are stimulated by the presence of some food material, such as crab meat, or by some substance containing potassium ions, the direction in which the cilia of the lips lash is reversed, so that particles are carried into the mouth rather than away from it. Such reversal occurs only on the part stimulated and there is no evidence of the spreading of the stimulus through nervous action. Also the wave of propagation of the ciliary movement as well as the ciliary stroke is changed in direction. Under these conditions it is a matter of interest that the direction of the stroke of the cilia of the tentacles and of the siphonoglyphes remains unchanged.

Undulating Membranes.—Many of the protozoans are provided with undulating membranes which serve as locomotor organs. These have long been considered made up of rows of fine cilia fused together into a homogeneous membrane. Chambers and Dawson have given a demonstration of the fact that this supposition is correct. They have shown by microdissection methods that it is possible to pierce the membrane with a very fine needle. When the membrane is so pierced it tends to break up into a series of fine cilia. So long as the needle is kept in position the cilia of the disrupted portion of the membrane repeatedly fuse and separate again. When the needle is removed the cilia fuse again into a definite membrane. The reconstitution of the membrane occurs by a fusion which spreads from the bases of the cilia towards their free ends, and is of such a character as to suggest the secretion by the cilia

of a slime-like substance which spreads over the cilia so as to join them into a homogeneous sheet.

The Graphic Method.—In order to gain some definite information as to the sequence of events occurring during a muscle contraction some form of graphic-recording apparatus is used by means of which the muscle makes upon a moving surface an actual record of its own contraction and relaxation. Such methods have been devised of a variety of forms, some crude, some highly refined and specialized. A very convenient form for common use is that in which the muscle attached to a firm support at one end has its other end attached to a lever rotating about a fulcrum. The free end of the lever bears a fine writing point and is so placed as to make a light contact with a smoked paper borne upon a rotating drum. As the drum rotates the muscle will trace a record of its contraction and relaxation. Used in combination with some form of time marker indicating appropiate intervals of time, and with the drum rotating at the proper speed, the record can be made to show the actual time relations of the different phases of contraction and relaxation.

The Fundamental Physiological Properties of Muscle.—The general physiological properties of protoplasm and the physiological division of labor resulting in the production of highly specialized forms of tissue adapted to do certain special forms of work have been considered. Now the behavior of one of these highly specialized tissues will be examined rather intimately, not alone because of its interest as a mechanism of movement, but especially because of its extreme exhibition of two or three of the fundamental properties of protoplasm, namely, irritability, conductivity, and contractility. As material for this study, striated muscle chiefly will be used, as giving the highest form of development of the irritable and contractile conditions and their attendant phenomena, and because this form of muscle has received most attention from investigators. It is more than likely that other forms of contractile tissue will, upon further study, be found to be just as interesting and illuminating.

Muscular Movements.—There is a wonderful variation in the degree to which the motor elements are found to be differentiated in different animal groups, and in different parts of the bodies of the higher animals. It is common for histologists, working chiefly upon the muscles of the higher animals, to recognize the existence of three forms of muscular tissue: (1) smooth muscle—non-striated, involuntary in its action; (2) cardiac muscle—striated and taking the form of a syncytium with very many nuclei; and (3) striated muscle—voluntary, and forming the bulk of the skeletal muscle of the body. This classification is inadequate when all of the motor tissues found in the animal kingdom are taken into consideration, but it has the advantage of applying very generally to those types of muscle cells most commonly known. There is a form of

cell, the pigment cell, which has a power of movement comparable in a way to that of the smooth muscle, and yet somewhat more primitive. In this case it is to be stated that movement is not the chief characteristic of the cell, and yet it is the property of movement which makes it possible for the pigment cells to serve as a protection to other cells from the action of light, or for the purpose of rendering the organism of which it is a part less conspicuous.

The different types of muscle mentioned differ not only in structure but in behavior. Striated muscle, in its normal condition, contracts with great rapidity and only upon stimulation; cardiac muscle is by some supposed to contract automatically, but the weight of evidence indicates that here also normal activity is due to stimuli coming from nervous centers; the smooth muscle, being less differentiated, appears to have retained more of its power of spontaneous contraction.

Distribution of Contractile Elements in the Animal Kingdom.—That an evolution of contractile structures has occurred is very readily seen when making a comparative study of the contractile elements in animals

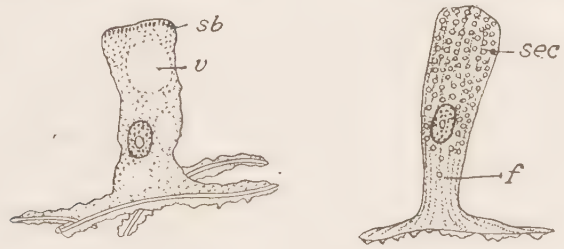


FIG. 67.—Muscle cells from the hypodermis of *Hydra fusca*. *A*, cell from oral region; *v*, vacuole; *sb*, striated border. *B*, cell from foot region, showing secretion granules *sec*, and protoplasmic strands.

of different phyla. Evidences of the arrangement of the protoplasm into contractile fibers appear even among the protozoa. Here the contractile elements are known as myonemes. These are usually considered ectoplasmic structures, lying in various directions in the protoplasm of the body, and serving by their contractions to modify the form of the body or to move it into another position. The accounts given of the structure and arrangement of these elements are far from satisfactory at present, but there is ground for hope that this matter will soon be made clear. The work of Kofoid and of his students MacDonald, Swezy, Sharp, Taylor, and Yocum upon the neuromotor apparatus of various of the protozoa opens up an entirely new field. Some of this work will be found reviewed in the section upon the nervous system.

Among the Porifera, muscles can hardly be said to exist. There are found epithelial cells, modified somewhat in shape through elongation, which are slightly contractile. Parker found that these cells arranged

about the oscula of the sponge Stylotella would close the osculum in about 7 minutes after stimulation.

Among the Cœlenterata there is found a variety of conditions. Such forms as Hydra possess neuromusculoepithelial cells (see Figs. 67 and 68). These serve at the same time as protective, receptor, and effector cells. In others of the same group there are found definite muscular fibers arranged in bands in the body walls, and while the action of these muscle cells is slow it is in all respects comparable with that of smooth muscle found in the higher animals. In certain of the Medusæ there is also to be found muscle which has become so differentiated as to give a slightly longitudinally striated appearance. This muscle is undoubtedly

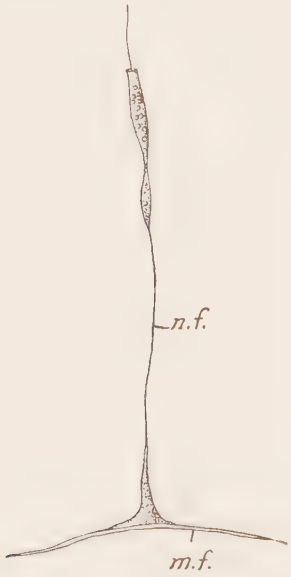


Fig. 68.—Musculoepithelium cell of *Anemonia sulcata*. *m.f.*, muscle fiber. *n.f.*, nerve fiber.

Fig. 69.—Muscle cell of *Cercariæum*. *c*, cell body. *m*, muscle fiber.

much simpler than the striated muscle of higher forms, and really represents but a slight advance in complexity of structure over that of the unstriated muscle fiber. In some of the Hydroids there is also found evidence of fibrillation of the musculoepithelium into rather definite processes.

Among the worms are found elongated, very faintly fibrillated, longitudinally striated, but not cross-striated cells (Fig. 69).

Among the molluscs there are examples of cardiac striated muscle. The other muscles of all except the highest forms are of the unstriated variety.

Among the Crustacea and Insecta both striated and non-striated muscles occur. The striated muscles of some of the Insecta are extremely complex in structure.

The higher animals show an abundance of striated muscle, but the visceral muscles are of the non-striated variety.

Structure of Striated Muscle.—A full understanding of the workings of such a tissue as striated muscle can hardly be expected without a knowledge of its more minute structure. Much time and effort have been expended by many investigators in an attempt to solve the riddle of muscle structure, but a complete and satisfactory knowledge of it is still to be obtained. The accounts given of muscle structure in most of the histologies are of little value to one who is making an attempt to explain the way in which the muscle operates.

Muscle fibers taken from different animals appear to vary considerably in their structure, and perhaps this is to be expected. Certainly, it would hardly be expected that a contractile tissue had reached the same degree of evolutionary differentiation in all phyla of the animal kingdom.

In general, it may be said that the skeletal muscles of an animal are composed of muscle fibers, which may be grouped into larger or smaller bundles. Under the high powers of the microscope each fiber appears made up of thread-like and regularly marked fibrillæ or sarcostyles running parallel to each other for the full length of the fiber. Closer inspection reveals the fact that the fibrillæ or sarcostyles do not constitute the whole of the fiber, but that they comprise a larger or smaller part of it, being immersed in a cylindrical or prismatic mass of rather liquid substance, termed sarcoplasm, bounded by a definite membrane, the sarcolemma, which adheres closely to the surface of the fiber. It has been held that the sarcolemma is really a product of the connective tissue, but this idea is now disputed and it seems that the sarcolemma is, in fact, a part of the muscle fiber, but at the same time most intimately associated with the connective tissue.

In addition to the fibrillæ or sarcostyles which lie in the sarcoplasm there are also numerous nuclei, slightly elongated in form and in other ways different from the nuclei of the connective-tissue cells lying in the cytoplasm between the fibrils and sarcolemma. Other nuclei are scattered about among the fibrils, rather deep down in the fiber. These nuclei do not appear to have any very definite significance so far as the cellular character of the fiber is concerned.

That the bundle of fibrils is really what it appears to be is easily shown, since these elements are very likely to become separated from each other and to stand out alone. In the muscle of the sucker such a fibril or sarcostyle is claimed by Dahlgren and Kepner to be composed of two kinds of substance, the so-called isotropic and anisotropic sub-

stances. These substances are distributed in the fibril with absolute evenness and regularity. The isotropic substance does not readily stain, while the anisotropic substance stains very deeply.

The anisotropic substance is deposited (in the sucker muscle fiber—see Fig. 70), in regular areas which are of equal length and equally spaced from each other. For convenience each of these is designated by the letter *Q*. Such a portion of the anisotropic substance with one-half of the isotropic substance on each side of it is commonly referred to as a sarcomere. This arrangement holds

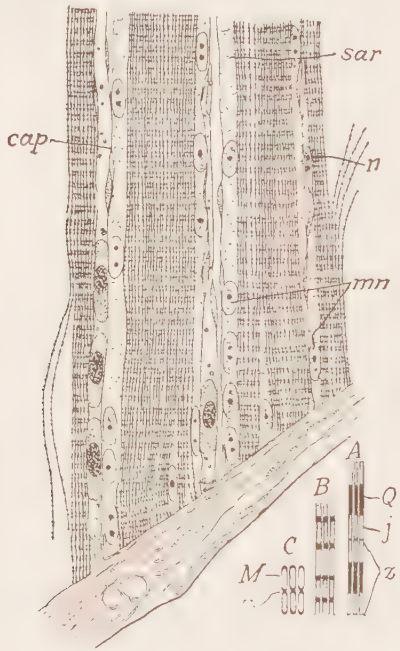


FIG. 70.—Longitudinal section of a bit of muscle from the sucker Catostomus. As the scale will not permit of fine detail, a semidiagrammatic sketch in the lower right corner serves to show the relations of dark and light elements in the three contraction stages of the fibril. The chief figure shows the *Q*-stripe separated as in *B*. *cap*, capillaries containing blood cells and blood platelets; *sar*, sarcoplasm; *mn*, muscle nuclei; *n*, connective-tissue nuclei. × 1000. (*Dahlgren and Kepner, "The Principles of Animal Histology." Copyright* 1908 *by the Macmillan Co. Reprinted by permission.*)

for the fibrils in the voluntary muscle of this form, and it is further true that where a number of fibrils are gathered together into a bundle the sarcous elements are all in perfect alignment and directly opposite each other. This gives to the fiber its striated appearance. In the case of the sucker the broad, darkly staining portions are about two-thirds the width of the intervening light stripes.

In most instances this band of the sarcous element is divided at its middle by a lighter band. This is caused by the beginning of the act of contraction in which the broad, black bands of the sarcous elements separate into two parts (Fig. 70*A* shows this condition).[1]

[1] Quotation from Dahlgren and Kepner, "Principles of Animal Histology." Copyright, 1908, by The Macmillan Company. Reprinted by permission.

The light or isotropic band of the muscle fibril, is seen on close inspection to be divided midway between two successive anisotropic bands by a dark line, the intermediate septum, *Krause's membrane,* to which the letter *Z* has been given. In any individual fibril the membrane is represented by a spot or granule. Dahlgren and Kepner and others hold that the intermediate granules in any single plane are bound together by a transparent membrane. That this is true seems to the present writer a matter of some doubt.

A suitable muscle for the study of the structure and relations of the fibrils is that found in the appendage of the lobster. The following account is taken from Dahlgren and Kepner's "Principles of Animal Histology":[1]

The muscle shown in the figure was from the basal joint of the antenna of a lobster that was on the point of emerging from its shell. This figure (Fig. 71) represents a part of the fiber, the upper end of which was attached to the shell. The two smaller nuclei are probably the first edges of nuclei that are, in reality, as large as the two larger ones.

The fortunate condition is that the part of the muscle next to the shell was at rest and in a relaxed condition, while all the other segments showed successive stages of contraction. The first segment measured, in one of its magnifications, 17½ millimeters. Each succeeding one measured a little less, until the last is only 12 millimeters long. This alone shows us that we have a carefully graded series of contraction stages, and an examination of the successive stages confirms this view.

In the first upper segment of the eight sarcous segments shown in any one of the sixteen myofibrils that compose this fibril bundle, we can notice that the anisotropic material is deposited in a long rod-like area in the middle of the segment. This area we will designate as *Q*, and it is usually so-called in works on muscle structure.

On each side of *Q* is an area of non-staining isotropic substance not more than a quarter as long (or wide, if we consider the whole band that they form) as *Q*. Conforming with usage, we shall call this *j*. There are two of these.

Bordering on *j* on its side farthest from *Q* is a row of dots or round bodies. These are of black-staining anisotropic substance and are called *Z*. They mark the limit of the sarcous element or muscle unit of the fibril, and where two elements touch, the dot is double, although the two parts are molded together and do not usually appear as twin bodies. The upper segment in our figure shows both a double *Z* body (where it touches the segment next lower) and a single or half *Z* body at its upper end, where it is attached to the connective-tissue cells.

The *Z* bodies, then, form the intermediate line and are connected with one another by a transparent or non-staining membrane that extends through the cell from side to side, even in such parts of it as contain no myofibrils. This can be well seen in the portion of the fiber figured, especially where the larger and smaller bundles of fibrils have separated to make room for the two large nuclei and their surrounding sarcoplasm.

[1] Quotation from Dahlgren and Kepner, "Principles of Animal Histology." Copyright, 1908, by The Macmillan Company. Reprinted by permission.

The second muscle segment shows no great difference from the first, although it is a trifle shorter. In the third, however, a change has occurred and *Q* becomes thinner in the middle. The whole segment is about one-twentieth shorter than the second. In the fourth segment the *Q* area is not only thinned out in its middle, but its substance does not stain as black in this thinned middle part.

The remaining five segments are alike in the fact that the *Q* areas are elongated until their black ends have met the corresponding ends of the *Q* areas of the neighboring segments. In doing this they hide the *Z* dots and make a much darker and wider band across the muscle fiber in its place. Their middle parts have become clear and non-staining, except that an edge appears to be left on each side. This is probably a refraction line.

In the middle of this *Q* area, now light and non-staining, appears a small black dot that resembles the *Z* body of the relaxed stage except that it is smaller and is not connected with its neighbors by a membrane. This dot with its neighbors forms the *M* stripe. (In Fig. 71 the *M* stripe is the same width as the two halves of the *Q* stripe.)

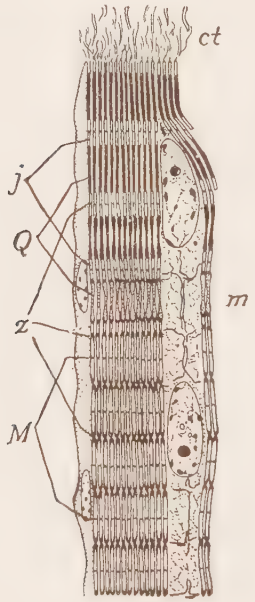


FIG. 71.

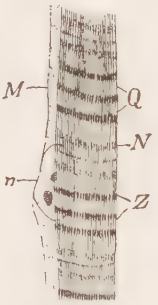


FIG. 72.

FIG. 71.—Structure of muscle of lobster. (*Dahlgren and Kepner, "The Principles of Animal Histology." Copyright* 1908 *by the Macmillan Co. Reprinted by permission.*)

FIG. 72.—Bit of muscle fiber from the body wall of an adult larva of Corydalis cornutis. Shows the *N* stripe near the Krause's membrane *Z*. *n*, nucleus. (*Dahlgren and Kepner, "The Principles of Animal Histology." Copyright* 1908 *by the Macmillan Co. Reprinted by permission.*)

The usual appearance of the fiber is now entirely changed, and upon a careless examination appears to have been reversed, as though the *Z* bands and *Q* bands had changed places. It takes a careful eye, even in such a favorable specimen as the one from which the figure was drawn, to run from band to band and note the real change. This difficulty is a weakness of the eye muscles and can be demonstrated by an attempt to count the pickets in a distant fence, which can be clearly seen if the eye remains still, but are lost count of if the eye moves to follow them.

Many other forms of muscle have a more complicated pattern of sarcous elements than the ones we have been studying, as in some insects, for example, where a band of anisotropic areas in the fibrils extends through the middle of

the *j* area. When found, this band is called the *N* band (Fig. 72). Its presence causes some unimportant complications in the contraction process. The isotropic bands are designated by small letters.

In most muscles, the bands are so fine and close set that it is difficult to distinguish them clearly. Then, again, one does not know what stage of contraction is presented. The usual condition shows one of the intermediate stages, as in the sucker muscle, where the eye of the ordinary observer would take each of the two ends of the divided *Q* stripe for the *Q* stripe of the resting muscle, until study showed the true relations.

Extensibility and Elasticity of Muscle.—One of the simplest observations to be made upon muscle, either when it is in its natural position in the body or when it is excised, is that it is capable of being stretched beyond its normal length, and that when the force which altered its form is removed it tends to return to its original form and length. It may therefore be said that the muscle fibers are extensible and that they are elastic.

The extensibility of muscle is somewhat unlike the extensibility of certain non-living substances, such, for example, as a rubber band. The extensibility of the non-living rubber band tends to be directly proportional to the weight and to the length of the body extended. The extensibility of the muscle differs in that the increase in length with each successive addition of weight is in a diminishing ratio. If the successive lengths of a frog muscle, as it is successively extended by additions of uniform weights, are represented by a curve, the curve approximates the form of a parabola (see Fig. 73). As the weights are successively removed the muscle shows a regularly increasing elasticity. Usually under laboratory conditions the muscle fails fully to regain its original length.

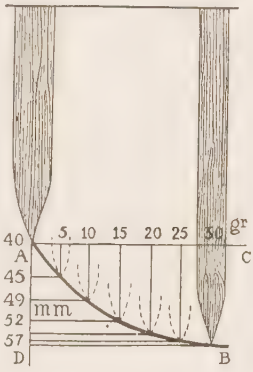


FIG. 73.—Curve of extension of muscle. This diagram illustrates the effect of increasing loads upon the extension of muscle. (*From Brubaker, after Gad.*)

These properties of extensibility and elasticity vary greatly under different conditions of activity, fatigue, and physiological condition. Muscles, as commonly experimented upon in the laboratory, are under exceedingly abnormal conditions, having been removed from their natural environment, frequently suffering from mechanical abuse, being deprived of their blood supply with a consequent lack of oxygen and accumulation of wastes, and having had their nervous supply seriously disarranged. There is little doubt that under normal bodily conditions muscles would show perfect elasticity, and that the departures observed from perfect elasticity are due to the abnormal conditions under which the muscles are working.

Muscle Action.—The study of the phenomena of irritability and contractility, like other physiological processes, may be either descriptive

or analytical. As descriptive physiology, some conception of the grosser facts and appearances of the various reactions might be secured, and the analysis should lead to some idea as to the nature of the fundamental processes involved in bringing about these appearances.

First, an examination may be made of some of the facts of irritability and contractility and later an attempt to arrive at some understanding as to how the appearances may be brought about.

Forms of Muscle Contraction.—Students of muscular activity recognize two types of muscular contraction, isometric and isotonic:

1. *Isometric Contractions.*—These are contractions in which the muscle, when stimulated, exerts its force against the immovable supports to which it is attached and thus result simply in the development of tension.

2. *Isotonic Contractions.*—These are contractions in which the muscle, when stimulated, exerts its force in moving a part in relation to another part to which it is attached and so accomplishes work. In this case the weight lifted must be applied in such a way that its inertia does not come into play.

A muscular contraction may be of one type during one part of its contraction and of the other type during another part. It may be reasonable to question whether any muscular contractions may not at first be isometric and then isotonic, as in lifting weights, etc.

The Muscle Twitch.—When a muscle is stimulated with a single induction shock either through its nerve or directly, it responds by a rapid contraction and relaxation. This form of contraction is not at all like the contractions which normally take place when the muscle is situated in the animal's body, being simpler in type. A study of this form of contraction will give information tending to make clear the normal mode of muscle action. In order to examine the series of events occurring during this contraction and relaxation, which will be referred to as a *twitch* (Fig. 74), the isolated muscle is set up in connection with a writing lever having its writing point in contact with the smoked surface of a rapidly rotating drum. Directly in the same vertical line with the writing point of the muscle lever is the writing point of a signal magnet which is introduced into the primary circuit of an induction coil which is to furnish the stimulating current. This writing point will then record the instant of completing and of breaking the circuit—and thus the instant of stimulation. A tuning fork beating $^1/_{100}$ second should also be provided for recording the time intervals actually occupied by the various phases of the muscle action. The drum is allowed to rotate rapidly, the key controlling the electric circuit is closed, the muscle contracts and relaxes, the key is opened, the muscle contracts and relaxes again, each time tracing upon the smoked surface of the drum a record of its movements. The record gives information as to the total

time required for the contraction and relaxation, the time required for the contraction and relaxation respectively, the time elapsing between the application of the stimulus and the beginning of the contraction, and the relative rates of contraction and relaxation during the different phases of each process. Also by measuring the height of the curve produced and knowing the length of the muscle lever involved, it is possible to calculate the actual shortening of the muscle, and then to determine the actual work done by the muscle.

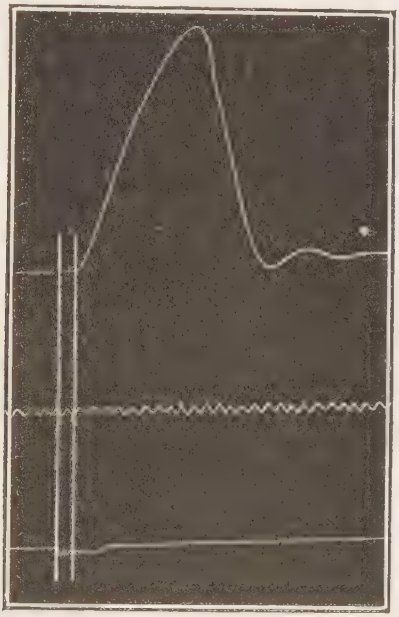


FIG. 74—Single muscle twitch—frog's gastrocnemius. The upper line is the record of the muscle contraction, the middle line is the record of a tuning fork beating $\frac{1}{100}$ second, and the lower line indicates the instant at which the electrical stimulus entered the muscle. The writing points of the muscle lever and the signal magnet were in the same vertical line. The distance between the two vertical lines gives a measure of the time elapsing between the instant of stimulation and the beginning of contraction, *i.e.*, the latent period.

The Latent Period.—Any record of the contraction of a muscle made upon a rapidly moving surface shows that the muscle does not contract immediately upon stimulation, no matter what be the strength of the stimulus. A certain small interval of time elapses between the stimulating impulse and the beginning of contraction. This period is known as the *latent period* (see Fig. 74). It might easily be thought that no change is taking place during this period were it not for the very definite evidence of an electrical change in the muscle beginning almost simultaneously with the application of the stimulus. This electrical change is an indicator of the excitation process of the muscle and appears to be

independent of the contractile process, since it is possible, as shown by Mines, completely to separate the two processes.

The change which occurs in the muscle substance following the application of the stimulus is, according to present evidence, a chemical one and apparently consists in the breakdown of a compound of sugar and phosphoric acid known as hexose-phosphate so as to yield lactic acid and phosphoric acid.

At all times the muscle substance is giving off small amounts of carbon dioxide; this small amount is not increased when the muscle is stimulated under lack of oxygen. This carbon dioxide is not the result of ordinary metabolism, but has been shown to be due to the neutralization of acid produced in the muscle under anaerobic conditions by the carbonates of the muscle substance.

Effect of Temperature upon Muscular Contraction.—The effect of changes of temperature in physiological activities is very clearly shown in muscle. All phases of the contraction are affected. Lowering the temperature to 0° or thereabouts decreases the irritability of the muscle, and lengthens the latent period, the period of contraction, and the period of relaxation. On the contrary, raising the temperature up to, say, 28°C. increases irritability, and shortens the latent period, the period of contraction, and the period of relaxation. At some point between 26 and 30° the muscle usually reaches its optimum activity. At temperatures above the optimum the activity of the muscle decreases rapidly. Not all muscles are affected in the same way by changes of temperature. The muscles of different species and of different individuals of the same species behave somewhat differently. In the case of frogs, the history of the animal previous to experimentation is a factor to be considered. The muscles of animals which have been kept for a long time in very cool surroundings behave differently from those which have been kept at summer temperature for some time.

The contraction of muscle involves both physical and chemical factors. It is not possible that all of these factors should be influenced in the same way or to the same degree by changes of temperature. Wooley has shown that the temperature coefficient of the latent period of the frog *Sartorius* is 2.0 for temperatures between 5 and 15°C. and 1.8 for temperatures from 10 to 20°. In general, the results of experiments indicate that the temperature coefficient of the whole period of muscle contraction and relaxation are such as would be expected if the muscle contraction is due to some fundamental chemical process (see Fig. 75).

Tonicity of Muscle.—The muscles of the living animal are normally at all times under a slight tension, that is, they are always, even when at rest, slightly stretched. Due to this fact, muscular responses after stimulation are more efficient than would be possible if the muscle fibers hung loosely between their attachments. It has been suggested that this

tone of the muscle fibers is of nervous origin. The fact that the muscles lose tone and become stretched out and more or less flabby when the nerves supplying them are severed points in this direction.

Another set of facts is also of interest here. Muscles bathed in pure sodium chloride solutions lose tone and become very much stretched out, even by a very light load. When stimulated, such muscles contract less effectively than normal muscles. Whether this harmful effect of the

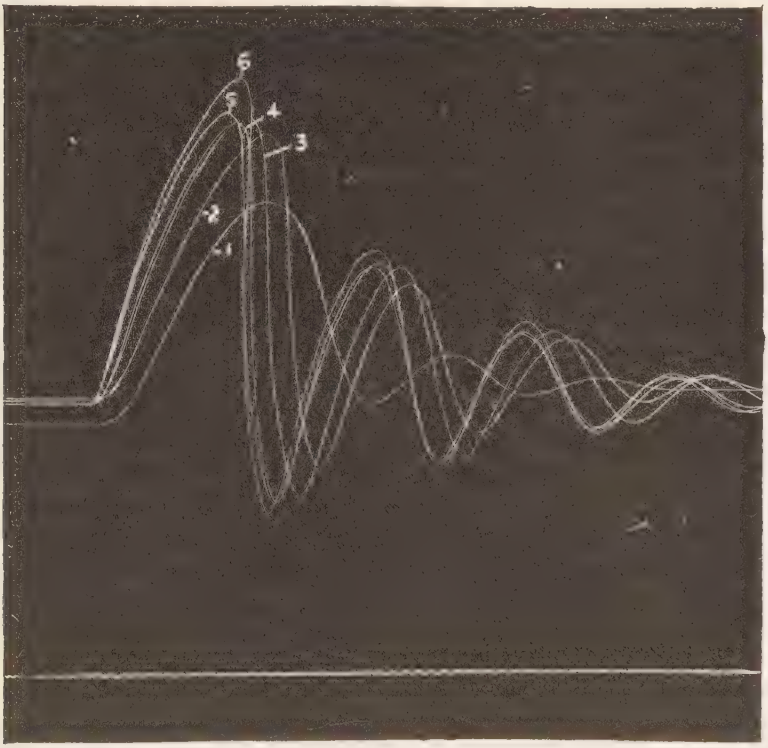


FIG. 75.—Effect of temperature upon the contraction of the frog's gastrocnemius muscle. 1, at 10°; 2, at 15°; 3, at 20°; 4, at 25°; 5, at 30°; and 6, at 35°. The temperature coefficient for the whole range is approximately 3.1.

sodium chloride is upon the muscle fibers directly, or upon nerve elements going to the muscle fibers, has not, so far as the author is aware, been determined. Tone may be restored to muscles which have lost their tone in sodium chloride solutions by immersing them in solutions containing calcium in addition to the sodium chloride. If there is an excess of calcium in the solution a greater tone, that is, a somewhat greater degree of constant contraction than normal, may be maintained. Constancy in the hydrogen-ion concentration of the fluid bathing the muscle is also an important consideration in maintaining normal tone.

Staircase Contraction. When a perfectly rested muscle is subjected to several stimuli at short intervals, it is found that in both skeletal and cardiac muscle the successive contractions induced by stimuli of uniform intensity are of regularly increasing amplitude until a certain maximum is reached. Mines regards the production of lactic acid in the muscle as responsible for the changes in surface energy and shows that, since there is an optimum hydrogen-ion concentration for the contractile response, the first effect of the production of lactic acid may be an increase of this factor from a suboptimal to an optimal value.

In a study of the *staircase* phenomena in the gastrocnemius muscle of the frog, Guenzberg found an interesting fact. When the muscle was perfused for a number of hours (6 to 24) with a Ringer solution lacking in potassium and the sciatic nerve was stimulated by induction shocks every 2 seconds, if a continuous current of oxygen was bubbled through the perfusing fluid there was a prolonged staircase effect, probably the result of the retardation of the metabolic processes necessary for the production of a sufficient amount of lactic acid by the presence of the oxygen. If the perfusing fluid was charged with carbon dioxide, there was a much more rapid ascent of the staircase.

Summation of Contractions and the Development of Tetanus. — The behavior of the muscle when stimulated by a single impulse has been seen. If, now, a series of successive stimuli is applied to the muscle at regular intervals, it will be found that the effect upon the muscle will vary according to: (1) the kind of muscle used; (2) the rate at which the stimuli enter the muscle; and (3) certain external conditions, such as temperature and the previous history of the muscle.

If the impulses enter the muscle at a rate which, after a contraction, allows the muscle fully to relax before the next succeeding impulse enters the fibers, there will be no change in the form or the character of the contraction, except that possibly successive contractions may show a gradually increasing amplitude of contraction, the so-called *staircase* effect. If, now, the interval is shortened between two successive stimuli so that a second stimulus is given to the muscle before relaxation from the first contraction has occurred, it is found that the muscle immediately (after the usual latent period) contracts, and this contraction is in part added to the original contraction, so that the total shortening of the muscle is considerably greater than would be caused by either stimulus alone. If, now, a third stimulus enters the muscle before the relaxation from the second has taken place, there is again an additive effect, and the total contraction of the muscle reaches a still greater value. With succeeding stimuli, the muscle continues to contract more and more until it reaches a certain maximum beyond which it is not possible for the muscle to go. This superposing of contractions one upon another is known as the *summation of contractions.* It has been found that the

greatest effect from the second stimulus occurs when it is applied during the last third of the contraction resulting from the first stimulus. On the other hand, if the second stimulus is applied during the latent period following the first stimulus, no extra contraction takes place. The second stimulus appears at this time without effect. During this period the muscle is said to be in a *refractory phase.*

If, now, the intervals between successive stimuli of the muscle are regulated so that they are somewhat less than the time involved in the total contraction and relaxation period of the muscle (see Fig. 76), it is found that when the stimuli are applied to the muscle it contracts, relaxes partially, contracts again, before the relaxation is quite complete, contracts again, and so on, the contractions and relaxations in each case being only partial, and when grouped together of the type referred to as

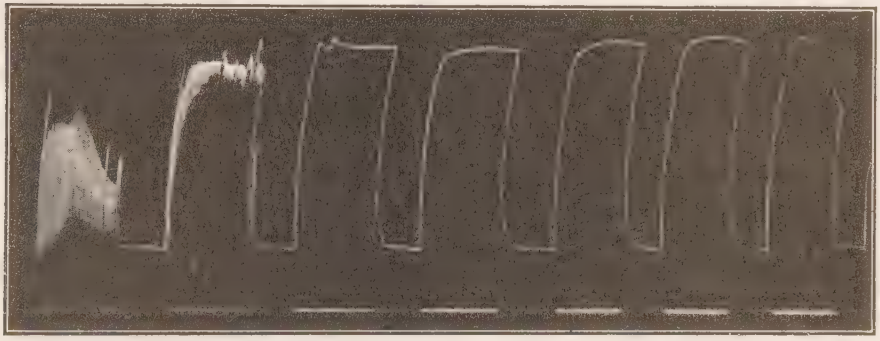


FIG. 76.—Genesis of tetanus in frog's gastrocnemius muscle. Number of stimuli per second, reading from left to right, are, successively, 2.4, 3, 3.5, 5, 7.2, 10.9, and 18.

partial tetanus or *clonus.* If, now, the rate of stimulation be increased slightly, it will be found that the relaxation phase will more and more disappear, and if the rate be increased to twenty to twenty-five impulses to the second, in the frog muscle, the contractions will become fused and the crest of the curve traced by the muscle will become smooth and regular, showing that relaxation does not at all take place following a given impulse before the next stimulus enters the muscle. This long-continued, maximal contraction is called *tetanus.*

The tetanic contraction will continue (within limits) so long as the stimuli continue to enter the muscle. It will be noted that the muscle is not, however, able to maintain the full degree of its contraction for a long time. The crest of the curve soon begins to decline. This is an indication of fatigue and is probably due to the effect of the gradually increasing concentration of hydrogen ions in the muscle substance. As the amount of lactic and phosphoric acids formed and held within the muscle fibers increases, it can readily be imagined to be above the optimum during tetanic contraction, and the effect of such excess is con-

tinually to decrease the amplitude of the successive twitches making up the tetanic contraction.

The number of impulses per second needed to throw a muscle into the tetanic type of contraction varies with different types of muscle, with different species of animals, and with muscles of the same species under different conditions. Stimuli entering smooth muscle at the rate of from five to seven per second may induce tetanic contractions. Stimuli entering skeletal muscle at the rate of two to three per second will bring about tetanus in the tortoise; at ten per second, in the muscle of the rabbit; at fifteen to twenty per second, in the muscle of the frog; at fifty per second, in human muscle; at seventy to eighty per second, for the muscle of birds; at 300 to 340 per second, in the muscles of insects.

The previous history of the muscle, particularly as regards temperature, may influence the rate at which tetanus will appear. Frogs which have been kept at low temperatures for a long time—winter frogs—may show tetanic contractions at a much slower rate of stimulation than frogs taken in the summer—even though the actual experiments are performed at the same temperature.

From what has already been said it will appear that sustained volitional contractions are of the same type as the tetanic contractions. There is a difference, to be sure, in the manner in which the muscles are stimulated, the experimental muscle being ordinarily stimulated by rapid induction currents, while the nerve in the body of the living animal is stimulated by nervous impulses. Piper has shown that in the human being the nervous impulses enter the muscles at the rate of from forty to sixty per second. At the same temperature as that of the human body the nerve cells of the tortoise apparently have approximately the same rate of oscillation, as determined by the string galvanometer, and therefore stimulate the tortoise muscles at the same rate.

The rate at which the nervous impulses enter the muscle appears directly proportional to the absolute temperature.

The All-or-none Law.— In order to get the best results from the contractions of skeletal muscles it appears essential that each fiber should act as a separate unit, and that there should be no immediate communication between adjacent muscle fibers. In smooth muscle and in heart muscle, excitation may pass from one fiber to another, so that waves of contraction may pass over the mass of muscle from a given point of stimulation.

From the work of Bowditch upon the heart muscle of the frog there was established the *All-or-nothing* principle.

An induction shock produces a contraction or fails to do so according to its strength; if it does so at all it produces the greatest contraction that can be produced by any strength of stimulus in the condition of the muscle at that time.

Keith Lucas has established the existence of the same law with regard to the contraction of striated muscle fibers. The same principle seems also to apply in the transmission of nervous impulses and in the work of gland cells.

It had for a long time been supposed that slight or minimal contractions of a muscle followed slight or minimal stimulations of all the fibers composing the muscle, while maximal contractions followed maximal stimulations of the same fibers. The work of Lucas and of others who have followed his lead indicates the probability that there is not a delicately graded series of nerve stimuli which may pass to a muscle and thus bring about the contraction of a longer or shorter portion of the muscle fiber. Minimal contractions of the muscles are now believed to involve only a small proportion of the fibers making up the muscle, these contracting to the full extent of which they are at the time capable, and that maximal contractions involve contractions of all the fibers making up the muscle.

Muscular Fatigue.—Striated muscle, subjected to repeated stimulation for a considerable period of time, gives evidence of fatigue through

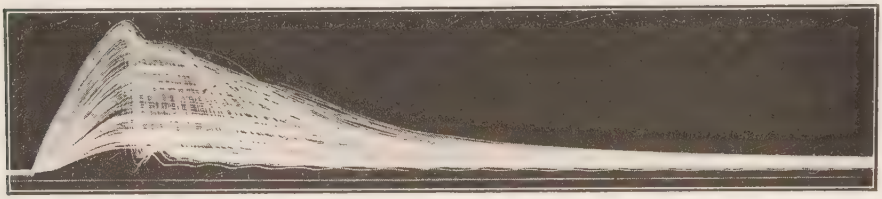


FIG. 77.—Fatigue curve of the gastrocnemius muscle of frog, taken on a rapidly rotating drum. Muscle stimulated once per revolution. All contractions recorded. Somewhat reduced in reproduction.

lessened amplitude of contraction, the development of a more or less permanent condition of contraction, called *contracture*, a prolonged latent period, prolonged contraction and relaxation phases, until there comes at last a time when the muscle is utterly unable to make any further contraction, no matter how great the strength of the stimulus. The condition of fatigue appears to involve both a loss of irritability and of contractility to a greater or lesser degree (see Figs. 77 and 78).

Fatigue appears to involve both a decrease in the energy-holding compounds available for the work of the cells and the accumulation of poisonous waste matters. Experiment easily demonstrates that an excised muscle becomes fatigued much more rapidly than one, in place in the body, in which the circulatory mechanism is still intact and functioning. One of the products of muscular activity is lactic acid. If this acid is produced at a greater rate than it can be removed from the muscle elements either by oxidation or by osmosis, the fatigue condition appears. In the excised muscle the fatigue appears the more rapidly because of the reduced amount of oxygen available for the oxidation of

the acid produced in the fibers as the result of those chemical changes leading to muscular movement. The acid produced is more likely to be the cause of the fatigue condition than the disappearance of the substances needed for muscular contraction, for Fletcher and Hopkins have shown that the amount of lactic acid produced in fatigue is only about one-half the amount produced when the muscle is subjected to heat rigor.

Substances other than lactic acid may have a part in the appearance of fatigue. Scott has shown that not only lactic acid but *b*-hydroxybutyric acid, potassium dihydrogen phosphate, and carbon dioxide stand as causal agents of fatigue. Certain products of protein disintegration, as indol, skatol, and phenol, may be active agents in producing normal fatigue. There is also some evidence that the negative ion of lactic acid and of *b*-hydroxybutyric acid, and that positive ions, particu-

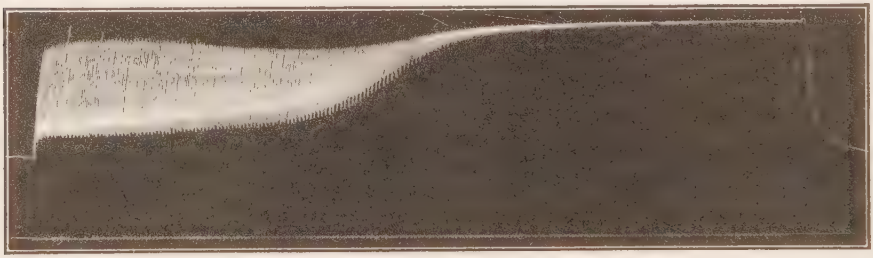


FIG. 78.—Contracture of frog's gastrocnemius muscle. Very slowly moving drum. Muscle stimulated once per second. Somewhat reduced in reproduction.

larly K′, are capable of producing certain fatigue phenomena. There appears no evidence at the present time that special fatigue substances, such as those proposed by Weichardt, exist, and there seems little probability that creatin or creatinine have any relation to muscular fatigue.

The Osmotic Pressure of Resting and Fatigued Muscle.—Professor Moore has given a very interesting and significant sidelight upon the events which occur during the fatigue of a muscle. He found by means of cryoscopic measurements that the average depression of the freezing point for the resting muscle of the bull frog is −0.42°C.; and that for the completely fatigued muscle it is −0.57°C. This difference is equivalent to that of a 0.08 molar solution of a non-electrolyte. The increased osmotic pressure of the fatigued muscle seems due to an accumulation of solutes of small molecular weight.

Determination of the Presence of Lactic Acid in Muscle.—The chemical substance involved in this increase in osmotic pressure is, in part at least, lactic acid.

F. G. Hopkins has originated a method which makes it possible to detect accurately the presence of lactic acid in muscle. The solutions needed for this test are: (1) a very dilute alcoholic solution of thiophene

(ten to twenty drops in 100 cubic centimeters of alcohol); (2) a saturated solution of copper sulphate; and (3) concentrated sulphuric acid.

The test is applied as follows: a glass beaker containing water boiling briskly should be ready at hand. Place about 5 cubic centimeters concentrated sulphuric acid in a test tube, together with one drop of the strong copper sulphate solution which serves to catalyze the oxidation that follows. To this mixture a few drops (the reaction being very delicate) of the solution to be tested are added and the whole well shaken. The test tube is now placed in the beaker water bath for from 1 to 2 minutes. The tube is now well cooled under a water tap, two or three drops of the thiophene solution are added from a pipette, the tube is replaced in the boiling water, and the contents immediately observed. If lactic acid is present the fluid, rapidly and in a highly characteristic manner, assumes a cherry-red color which is permanent only if the test tube be cooled immediately after the color appears.

Cooling, as described, should always be carried out before the addition of the thiophene, as the gradual appearance of the color, which then reappears on rewarming, makes the test much more delicate.

The test may be applied to frog's muscle as follows: A frog is pithed and afterwards allowed to remain quiescent for half an hour or so, care being taken that the circulation in the hind limbs is not impeded by pressure or flexion. Both hind limbs are then removed and the muscles of one, immediately after cutting from the bones, are rapidly ground with sand under 20 to 30 cubic centimeters of ice-cold alcohol. The other limb muscles may then be stimulated or caused to go into heat rigor, after which they are ground under alcohol. The alcoholic extracts from each limb are evaporated to dryness and the residues rubbed up with a few cubic centimeters of hot water. To each aqueous extract a decigram or so of finely powdered charcoal is added; each is heated to boiling in a test tube and filtered. The filtrates are evaporated in small glass basins and each dry residue is dissolved in 5 cubic centimeters of strong sulphuric acid and the acid transferred to a test tube for the application of the test as described. Not more than two to three drops of the thiophene solution should be added after oxidation. The resting limb usually gives a negative reaction, the extract from the other yields a brilliant color.

Heat Production in Muscle.—In the study of muscle a number of interesting facts have been mentioned in regard to muscle action. Such facts have a certain importance of their own, but they come to have a greater significance when in some way it is possible to relate them to some of the deeper, underlying considerations of how or why the muscle behaves as it does. The work upon muscle, interesting as it may be, cannot have in it much of real value unless it gives information of a more subtle character in regard to life processes. There is danger that too

much attention may be given to the more spectacular appearances and too little to the more fundamental facts of muscle action.

In the study of muscle action, as well as in the study of the actions of other types of protoplasmic cells, great difficulty has been experienced in coming to an understanding of what really happens in a muscle fiber when it contracts. It has already been pointed out that too little information has been obtained as to the structure of the muscle elements. This lack of knowledge affects an understanding of muscle action. Our most reliable information as to the nature of the processes which take place in muscle contraction comes from the work of Hill, Fletcher and Hopkins, and Peters.

Up to a comparatively recent time, the views of Hermann and of Pflüger upon muscle action have held sway. They were of the opinion that the discharge of energy by a muscle cell depended upon the dissociative breakdown of some labile molecule (Hermann's inogen) in which oxygen had taken its place beforehand (intramolecular oxygen) in such a manner that upon the dissociation of the molecule the energy yielded was energy due to a combustion, and the final products, carbon dioxide and water, represented the results of the combustion. It has already been noted that lactic acid is one of the products of muscle action. This lactic acid, which was supposed to arise from the same labile complex, has been variously regarded as either another final product, or as an intermediate product destined possibly to be used in the reconstruction of the inosit complex, or again as marking only a stage of incomplete combustion due to an insufficient oxygen supply. This theory has been based upon two main lines of evidence. The first is that the discharge of energy by a muscle and the yield of carbon dioxide are alike independent of the immediate oxygen supply (within wide limits). The second, according to Hermann, is that rapid heating fixes the inogen molecule from which lactic acid and carbon dioxide both came, and that a muscle rapidly heated gives off no carbon dioxide, just as it yields no lactic acid. Hermann further believed that the carbon dioxide of heat rigor, which he showed was diminished by previous fatigue, was so diminished not because carbon dioxide had been driven off from the muscle during the fatigue, but because the store of inogen from which it came in heat rigor had already been called upon and diminished by previous contractions. Pflüger also took the same two bases for his idea of intramolecular oxygen, in which he thought of the active substance of the muscle as being of the nature of a highly complex molecule after the order of Verworn's biogen and having intramolecular oxygen as one side chain and some oxidizable group at another place on the molecule as another side chain. He interpreted the increased production of carbon dioxide upon heating as evidence of an increased dissociation of the previously oxidized precursor and gave evidence to show that the carbon dioxide yielded by warmed

muscle was in reality newly dissociated carbon dioxide and not merely an expulsion of carbon dioxide already present in the muscle as such.

The more recent work has pointed to the view that lactic acid as such appears and disappears in the muscle as an incident of the machinery of muscle contraction, in which it or its hydrogen ion plays an essential part. The lactic acid appears to come from some substance referred to as the *lactic acid precursor*, *lactacidogen*, or *hexosephosphate*, and through its change of position effects a change either in the surface tensions or osmotic relations of the colloidal mechanism making up the muscle structure. The carbon dioxide arises from some molecular system, not necessarily the same as that concerned in the lactic acid formation, where a combustion by immediate and not antecedent oxidation supplies the energy for the restoration of the lactic acid to its former position in the precursor, and for the restoration of the status in *quo ante* of the muscle as regards its physical and chemical properties. It is to be noted that in this case the carbon dioxide is not yielded as the result of a dissociation of a molecule already containing intramolecular oxygen but appears always as the result of a direct combustion due to a contemporary supply of oxygen, except when in much smaller amounts it may appear from a muscle saturated with it, whenever circumstances make it possible for lactic acid to accumulate in the muscle to such a degree as to displace it from some physical or chemical combination. This expulsion of ready-formed carbon dioxide from the muscle tissues by increase of acidity provides an obvious interpretation for the fact that a muscle deprived of oxygen may nevertheless continue to yield carbon dioxide.

It will be recalled that two possibilities are before the muscle when it is stimulated. It may contract, in which case the energy liberated will be expended in doing muscular work, or it may simply develop a state of tension, a condition in which there is no shortening of the fibers and therefore no work accomplished. In this case the potential energy lost appears in the form of heat. Always also heat is produced during the restitution phase, and this may appear during a considerable period of time following the stimulation of the muscle.

The work of Hill and of Peters has given us the best information concerning the heat evolved during the energy transformations of the muscular work, although the problem has received the attention of many of the most able investigators. The study calls for a very high degree of technical skill in physical measurements.

When a muscle is stimulated directly, or indirectly through its nerve, in an atmosphere of oxygen, either by a single induction shock or by a short tetanizing current, it is found that heat production continues for some time after the mechanical response is over. It is found also that the amount of heat liberated after the contraction is over is at least as much as that liberated during the contraction itself. Careful experiment

leads to the conclusion that the delayed heat production is associated with an oxidation process occurring in the recovery of the muscle to a condition similar to that in which it was before the contraction took place. That oxygen is essential for this process is shown by the fact that this recovery cannot take place in the absence of oxygen, for example, in an atmosphere of nitrogen. Hill suggests that the process of muscle contraction is due to the liberation of lactic acid from some precursor, and that the lactic acid increases the tension in some colloidal structure of the tissue; that the lactic acid precursor is rebuilt after the contraction is over in the presence of and by the use of oxygen, with the evolution of heat; and finally that the heat liberated by a muscle excited in the absence of oxygen is due simply to the breakdown of the lactic acid precursor, and is the same in nature as the production of heat rigor.

When a frog muscle is stimulated to the point of fatigue by a tetanizing current in atmospheric air, Peters found that the heat production was approximately 0.9 calorie per gram of muscle tissue. By calorie is meant the amount of heat required to raise 1 gram of water 1° Celsius in temperature. The curve of heat liberation is roughly an exponential curve and shows that between 70 and 80 per cent of the total heat liberation occurs within the first 2 minutes of tetanus. This amount is about one-half of that of the heat produced by chloroform poisoning, for which a value of 1.70 calories has been obtained. If, after a muscle has been indirectly stimulated to the point of fatigue, it is then subjected to chloroform poisoning the heat developed in the chloroform rigor amounts to approximately 0.87 calorie per gram. From this it is apparent that the sum of the heats produced during contractions to the point of fatigue and the heat produced after the fatigue by chloroform rigor is about equal to the heat production of fresh muscle by chloroform rigor. This result is confirmation of the view that no process of importance occurs in the process of heat rigor which is not also involved in muscular contraction. Estimates of the amount of lactic acid produced in the heat measurements agree with those made under conditions of fatigue and rigor. Therefore, it may be said that heat production and lactic acid liberation are extremely intimately related.

When a muscle is allowed to shorten, in a tetanus of given duration and stimulus, the heat production is very much less than when the contraction is of the isometric type. The amount of the decrease of the heat-production value (which may be as much as 40 per cent) for an isometric contraction is of the same order as the percentage decrease in the length of the muscle. The greater the shortening allowed the greater the decrease in heat production will be. It is concluded, therefore, that the heat production is determined by and is proportional to the length of the fibers during activity, and that the heat production is a function of surface and not of mass.

An increase in the frequency of excitation, up to the limit when complete tetanus results, never decreases but almost invariably increases

SUMMARY OF CHANGES TAKING PLACE DURING MUSCLE TWITCH

Stimulus applied	Resting muscle—contains glycogen and hexose phosphate
Latent period	Hexose phosphate breaks down and liberates lactic and phosphoric acids
Contraction and fatigue phase	Chemical energy (potential) transformed to mechanical potential energy, then becomes kinetic, probably taking effect at colloidal interfaces. Elasticity of fibers altered. Tension developed in proportion to amount of acid produced anaerobically—about 400 calories of heat given off per 1 gram of lactic acid produced. Acid accumulates in muscle so long as recovery phase is delayed.
Relaxation and recovery phase, the latter continuing beyond the relaxation. Recovery means restoration to previous physicochemical condition.	Under aerobic conditions: Relaxation rapid, followed by recovery. Lactic acid removed from muscle fiber: *a.* By diffusion and subsequent appearance in blood as sodium lactate. *b.* Rebuilt through an oxidative process into hexose phosphate and possibly glycogen. Heat developed during recovery process about equal, or slightly in excess of that of contraction—about 500 calories per 1 gram of lactic acid transferred to original condition. Under anaerobic conditions: Relaxation may be prolonged. No recovery. Breakdown of hexose phosphate is irreversible.

sometimes very largely heat production. After complete tetanus is reached the heat production per unit of tension developed and maintained is absolutely independent of the frequency of excitation between

the limits of 17 and 100 per second. The tension development and the heat evolution are features of identically the same reaction.

The absolute value of the heat production in tetanus of the frog *sartorius* or *semimembranosus* is relatively constant at 15×10^{-6} calories reckoned per centimeter length of muscle, per second maintenance of tension, per gram weight of tension developed. Including the recovery processes of oxidation following the contraction this amount should be increased to 25×10^{-6} calories. This quantity, which may be regarded as the inverse of the efficiency of a muscle for maintaining a prolonged tension, is enormously large. The potential energy possessed by a muscle of 1-centimeter length when maintaining a tension of 1-gram weight is about $\frac{1}{6}$ gram-centimeter, or about 4×10^{-6} calories. Thus, in order to maintain a state of potential energy in a sartorius muscle about six to seven times that energy must be liberated per second. This extraordinary inefficiency and wastefulness of prolonged tension contrasts curiously with the high efficiency of the same muscle in a rapid twitch.

Red and White Muscle.—Dorothy Noyle Needham has reviewed the literature upon the differences between red and white muscle. Attention has been called by numerous investigators to the fact that muscles vary greatly in color, some being white, others containing more or less of pigment, so that they become even very dark red in color. The fact that color differences may be associated with differences in behavior has been appreciated since the work of E. Ray Lankester in 1871, who suggested that red pigmentation is found in muscles from which the most persistent and prolonged activity is required. Among the lower animals most of the muscles (except the heart) are not pigmented; among mammals most of the muscles are pigmented.

Ranvier discovered that more stimuli per second are required to produce a smooth tetanic contraction with white muscle than with red—in other words, the muscle twitch is slower in red than in white muscle.

Many histological studies of striated muscle have been made in the hope of correlating differences of structure with differences of function. The structural differences have largely to do with the presence or absence of granules in the sarcoplasm, or the presence or absence of hæmoglobin in the muscle fibers. The evidence presented by the different investigators is very conflicting, but upon the whole seems to indicate that:

1. The white fibers are the more irritable and give the higher twitch.

2. The red muscle fibers are the less irritable, give a greater contraction in tetanus than do the white, and can maintain the contraction for a longer period than can the white fibers.

Fisher made a study of the soleus (red) and the gastrocnemius (white) muscles of the cat and found that:

1. There is no difference in the length of the latent period.

2. The twitch of the soleus is more prolonged and slightly higher than that of the gastrocnemius.

3. The tetanus of the gastrocnemius is less marked than that of the soleus and the maximum shortening is obtained only with a much greater frequency of stimulation.

4. The tension curve for the isometric twitch of the soleus is prolonged, but lower than that of the gastrocnemius.

5. Tension is maintained for a longer time in the soleus than in the gastrocnemius.

Hunter, Hunter and Royle, also Latham, have given evidence in favor of the view that the skeletal muscles of the vertebrates consist of two sets of fibers:

1. A slender, granular type, maintaining plastic tone, chiefly involved in the maintenance of posture, innervated through the sympathetic, and in which lactic acid forms more rapidly but of less concentration than in the other type.

2. A large, clear type, concerned primarily with contractile tone, and, therefore, with movement, innervated by somatic nerve fibers, and which upon activity becomes more acid than the white fibers.

CHAPTER XXVII

EXCRETION

By *excretion* is meant the separation and elimination of waste materials from the body. These wastes may be of several sorts and they may vary somewhat from animal to animal and in the same animal from time to time. The variations are in part due to differences in the food supply and in part to differences in the metabolic processes carried on in the animal bodies. The wastes may be substances taken in with the foods or as foods but not absorbed or assimilated by the protoplasm of the body, substances which are either useless or actually harmful, or they may be products of cellular decomposition, such as carbon dioxide or uric acid, or they may be substances of a poisonous nature which are formed by the cells of the body as the result of normal metabolic processes or as the result of some pathological condition within the body.

In plants there are not, as a rule, special organs for the elimination of wastes, but these waste matters are stored either within the cells or in the walls of the cells. Among animals, on the other hand, special organs are usually provided for the elimination of wastes. In some of the lower forms of animals wastes are eliminated through the body surface only. There seems to have been a very gradual development of the power of the animal protoplasm to eliminate wastes—and a corresponding development of the structures necessary to do this work. In certain cases it seems quite likely that the work of certain eliminative structures was originally something else, and that the organ has assumed a selective glandular function of an eliminative character. It is true that, wherever found, excretory organs have another function, *viz.*, the regulation of the water content of the body, and thus the osmotic pressure of the liquids of the body. This is accomplished in one of two ways: either through a regulation of the excretion of salts from the fluids of the body, or through a regulation of the elimination of the superfluous water contained within the body. It seems to the author entirely possible that the original purpose of the excretory apparatus was either to regulate the water content of the body, whether uni- or multicellular, or to insure the rapid diffusion of water through the membranes of the body. Such diffusions would provide for constantly renewed supplies of such dissolved substances as might occur in the water, including oxygen. The utilization of the outflow of water from the cell as a means of getting rid of wastes may well have been at first a secondary matter, but in the higher animals at least it has come to have predominant importance.

Excretion among Protozoa.—The methods employed by the protozoa for excreting wastes vary according to the complexity of the animal and its habit of life. The forms which are endoparasitic or which are marine generally lack special excreting organs, though some of the marine forms resemble the freshwater forms in being supplied with contractile vacuoles. These serve as excretory organs—and possibly for other functions as well. The endoparasitic and marine forms live in a liquid of fairly high osmotic pressure, and so are not subject to the same stress of endosmosis which characterizes the forms living in the fresh waters. They, therefore, do not have the same need for a contractile vacuole as do those forms living in less concentrated waters.

In some protozoa (Ciliata, etc.) the contractile vacuoles appear fairly definite and permanent structures, while in others they appear temporary. In some forms the contractile vacuoles appear to be formed by the flowing together of small drops of watery liquid so as to form a larger drop which is definitely separated from the active protoplasm of the body by a membrane; in others (Paramœcium) there appear definite canals with constant position through which the liquid pours into a centrally placed vacuole having a definite wall. There is no doubt that the contents of these vacuoles are extruded to the outside. This is accomplished either through a break in the pellicle of the vacuole where it comes into contact with the body wall, or through a definite pore (Ciliates). The extrusion of the liquid can readily be observed by placing protozoa in a rather thick suspension of finely ground India ink, when the clear liquid of the vacuole will displace, at the boundary of the body, the thick ink suspension. Question has been raised as to whether all of the liquid contained in the vacuole is extruded to the outside. The evidence available indicates that all the liquid is so extruded.

R. A. Young has recently studied in sectioned material the structure of the contractile vacuole of Paramœcium. He finds that in this form the contractile vacuole, its pore, and its feeding canals form a continuous, permanent, ectoplasmic structure, and that it is not to be considered as dependent upon any sol-gel reversibility as has been suggested by Taylor in Euplotes. To this may be added the evidence submitted by Ruth B. Howland that the excretory apparatus of *Amœba verrucosa* and of Paramœcium is a definite structure. In the latter case it was possible to inject the vacuole and its tributary canals with dilute alizarin blue, and then to dissect it completely out of the animal.

The contractile vacuole serves not only as a means of eliminating surplus water, but also to eliminate whatever carbon dioxide is dissolved in that water. Whether other organic matters are also thrown off in solution remains to be seen, though it is claimed that the structure does eliminate some non-volatile substances.

The rate at which the vacuole does its work varies in different forms and under different conditions. Maupas has shown, for example, that *Euplotes patella* at 25°C. passes through its vacuole to the outside a volume of water equal to the volume of the body in 14 minutes, 16 seconds. *Paramœcium aurelia* at 27°C. accomplishes the same result in 27 minutes, 46 seconds, and Stylonichia at 24°C. the same result in 20 minutes, 28 seconds. The rate of contraction of the contractile vacuole is also influenced by temperature. The results obtained by different investigators differ in the temperature coefficient found. In general, the indications are that the reactions involved are more likely to be physical than chemical in character, though some authors speak of the temperature coefficient as being of a value similar to those known for chemical reactions. The following figures indicate the general character of the results obtained in this line of work:

	Seconds between Contractions	
Euplotes patella at 5°C.	61.5	
		$Q_{10} = 1.65 - 1.7$
Euplotes patella at 10°C.	48	
Euplotes patella at 20°C.	28	
Euplotes patella at 24°C.	23.5	
Stylonichia at 5°C.	18	
		$Q_{10} = 1.65$
Stylonichia at 10°C.	14	
		$= 2.15$
Stylonichia at 20°C.	6	
		$= 1.8$
Stylonichia at 27°C.	4	

There is some doubt as to the method of filling the vacuoles. Some hold that the membrane about the vacuole is continuous, and that the water diffuses through this as it would through any other permeable membrane. Upon this supposition it would appear that the osmotic pressure of the liquid within the vacuole must be greater than that of the liquid outside, therefore dissolved substances, presumably wastes, must be present. Carbon dioxide is, of course, present, and it is a substance which gives osmotic pressure to the liquid in which it is dissolved. Also it is suggested that the rate of contraction of the contractile vacuole is related to the rate at which carbon dioxide is produced in the body—and this is certainly chemical in character. It does not seem likely that salts are eliminated to any large degree through the contractile vacuoles.

The contractile vacuoles throw off from the cells not only water, but also a variety of substances soluble in the water passing through the cells. It would hardly be expected that the liquid emerging from the vacuole would be sufficiently concentrated in dissolved substances of any sort to make an analysis of the liquid an easy task. The contractile vacuoles surely dispose of the dissolved crystals which from time to

time are found in and disappear from the protoplasm. There is evidence that certain colored substances ingested by the cells are partly dissolved in the protoplasm in the vicinity of the vacuoles, and the color is diffused into the content of the vacuole, from which it is then eliminated from the body. There is also evidence that under certain conditions the content of the vacuole is acid in reaction (when stained by hæmatoxylin). This might indicate the presence of carbon dioxide as an important constituent of the liquid.

In addition to the work of the contractile vacuoles the protozoa have another method of disposing of wastes, *viz.*, the formation of crystals or granules which may remain included within small vacuoles in the protoplasm. Such inclusions have been found in individuals representing almost all classes of the protozoa. These bodies are of varying forms, are variously distributed throughout the protoplasm, and are possibly variable in their composition. Some of the earlier suggestions were that they were uric acid or calcium phosphate. The latter suggestion has met with the more general approval. The crystals and granules are built up during periods of abundant nutrition and decrease during periods of starvation. In starving Paramœcia, for example, the granules come to lie directly in the protoplasm instead of being held within vacuoles; they then gather about the contractile vacuoles, finally coming to lie very close to the boundary of the vacuole. The crystals decrease in size, disintegrate, and their substance disappears. The impression prevails that the dissolved material diffuses into the contractile vacuole and is thus eliminated from the organism.

Howland has found that the murexide test is not generally applicable to the detection of uric acid in the protozoa. Benedict succeeded in detecting the presence of uric acid in the fluid of *Paramœcium caudatum* cultures to the amount of 4 to 5 milligrams uric acid per liter by means of the Benedict blood-filtrate test.

Excretion among Metazoa.—Among the multicellular animals there are to be found, as might be expected, a great variety of excretory organs and of excretory processes. In the lower members of this group the excretory organs and excretory processes are very little differentiated, while in the higher forms the differentiation has reached a high degree of complexity. In most cases the excretory organs are specializations of cœlomic epithelium. In a somewhat sketchy way the story of increasing complexity of structure and function will now be indicated, passing in review from the simpler to the more complex.

Sponges and Cœlenterates.—Among the sponges and Cœlenterates there are to be found the simplest of the excretory functions. There are no especially developed excretory organs to be found within these groups, though in some of the higher members of the Cœlenterates certain structures take on an excretory function.

Superficial Intracellular Excretion.—In most cases there is to be found an excretion of wastes from the exposed surfaces of the animals. Such excretions are, of course, not very readily observed. Nor is it at all easy to determine just what substances are eliminated. Cotte has determined the fact that ammonia compounds are given off from some of these forms, while tests for uric acid, urea, taurin, tyrosin, and leucin have, in general, failed. While dissolved wastes may be eliminated by diffusion through the various cell membranes, there is little or no evidence that such elimination does occur. On the other hand, there is abundant evidence of the excretion of waste matters in the form of minute solid particles. These particles are not extruded from the surfaces of all body cells, but are carried to the outside by certain amœboid wandering cells which are derived from the mesoglœa and which, after leaving the body, disintegrate and free the excretory matters contained within them. Such excretion is referred to as *intracellular excretion*. This form of excretion is typical of such forms as Grantia and Reneira.

Storage Excretion.—In *Alcyonium* Fr. Pratt observed that certain mesoglœal cells exhibited a phagocytic behavior with respect to carmine grains. She believed that these cells would wander out into the gastric lumen and there dispose of their burden of foreign material, but she never actually observed that this happened. On the contrary, she had seen them round up, lose their pseudopodia, and for a short time at least remain within the tissues of the animal. The suggestion has been offered that this may be the beginning of a sort of localized *storage excretion*, in that these deposits of waste substances may remain within inactive cells. It can surely be believed that the storage of such materials in a single cell or a stratum of cells—or an organ—in parts of the body outside of the direct line of the more active metabolism may serve as a means of removing than from the more active cells. This process comes to be very similar to the storage of crystals and granules in the cells of many of the protozoa.

Localized Excretion.—Certain Cœlenterates show the deposition of crystals or granules in very definitely localized areas. This may be considered a sort of transition stage between the more generalized and not definitely localized condition described above, and that in which a definite glandular separation of waste products may be found. The case of Porpita mediterraneum is an illustration. This Siphonophore has under the border of the central organ a structure completely permeated by endodermal canals. This structure appears as a milky-white plate and contains a mass of crystals, small needles, and rhombic plates. The crystals are deposited in what appear to be ordinary endodermal cells. That is, an endoderm is found here, in a very limited locality, which takes on a definitely excretory function that of the deposition and storage of crystals. The crystals have been thought to be guanin. Whether

this is true or not, it is certain that some end product of metabolism, and therefore of excretion, has to be dealt with.

Glandular Excretion.—True excretory organs appear among the Hydromedusæ and Discomedusæ. Among the Hydromeduasæ there are found excretory pores opening from projections which occur on the subumbrellar portions of the bases of the tentacles. These pores lead back to the marginal canal, and through it to the radial canal, and through these to the stomach cavity. There is thus made a connection between the stomach and the outer medium. These pores do not, however, function as anal openings, but carry off to the outside pigmented granules and concretions of a waste character formed in certain specialized endodermal cells lining the canals.

Somewhat similar structures are found also among the Pneumatophores. It will be recalled that in these animals there is a great division of labor on the part of the individuals composing the colony, so that some serve as floats, some as motor organs, some as feeding individuals, some as protective structures, some as sensory structures, etc. The cavities of the so-called tentacles are in communication with the gastric cavities of the nourishing individuals. The main portion of the tube is shut off from the terminal portion which bears an excretory pore. Through this pore excretory matters are thrown off, hence Bedot proposes to consider the tentacles as the excretory individuals of the colony—although they doubtless have other functions to perform.

Excretion among Echinoderms.—The body fluid of the Echinoderms is very similar to sea water. It contains salts in solution, but little or no protein except that found in the cellular elements of the cœlomic fluid. This fluid must of necessity receive various end products of metabolism, and while such substances naturally occur in small amounts because of the slow rate of metabolism, there have been found small amounts of urea or some closely similar substance in the cœlomic fluids of Arbacia, Strongylocentrotus, Echinus, Sphærechinus, and Holothuria. The amounts of urea found are very small, being of the order of 2 to 4 milligrams per 100 cubic centimeters of cœlomic fluid in the sea urchin, and 1 milligram per 100 cubic centimeters of fluid in Holothuria. In addition to this there was found in the sea urchin 5 milligrams of some nitrogenous substance per 100 cubic centimeters, which was thrown off by centrifuging the liquid, an amount 2 milligrams in excess of that existing in the sea water.

The disposal of excretory materials may occur in one of the following ways:

1. By diffusion through respiratory or digestive membranes or, in some cases, superficial epithelium.
2. By intracellular excretion.
3. By storage.

Excretion by Diffusion.—There are no true excretory organs among the Echinoderms, hence excretion must be largely by diffusion. It would certainly be natural that, in those forms which are possessed of such elaborate skeletal frameworks, excretion should occur through those structures which also provide for gas exchange. In the different groups of the Echinoderms these are: (1) respiratory papillæ (starfishes); (2) oral branchiæ (sea urchins); and (3) respiratory trees (Holothurians). All of these structures involve cœlomic epithelium as one element of their structure.

The *respiratory papillæ* of the starfishes are hollow structures, like the fingers of a glove, which project from the surface of the animal. They are composed of two layers of epithelium, the outer or superficial ectoderm and the cœlomic epithelium inside of it. There are also connective-tissue cells and muscle fibers between the two layers of epithelium which make it possible for the organ to expand and contract. By means of this structure the cœlomic fluid is placed in close approximation to the sea water and diffusion of gases in either direction—and also of dissolved substances—is made easy. These papillæ are also provided, on their inner surfaces, with cilia which have the power to keep up a continuous circulation of fluid within themselves—and also in the cœlomic cavity. The *oral branchiæ* of the sea urchins are somewhat more elaborate structures and limited in their distribution to the oral membrane, but have fundamentally the same arrangement of tissues. They are much-branched, hollowed-out growths from the oral membrane and connect through the periœsophageal sinus with the cœlomic cavity. The *respiratory tree* of the Holothurians is an entirely different structure—so far as origin is concerned—from those just mentioned. It is a very much enlarged and branched diverticulum from the cloaca. This contracts and expands rhythmically and takes in and expels water through the anus. In all of these animals it is to be noted that there is the same fundamental arrangement of tissues separating the sea water from the cœlomic fluid—two layers of epithelium having between them a basement membrane made up in part of connective tissue and muscle cells. And it seems to the author a matter of some significance that this type of structure persists throughout the whole animal kingdom as the fundamental structure of a gland.

Through this respiratory membrane may occur not only the diffusion of soluble matters, but also an elimination of solid particles and cellular elements, although Henri and Lalou claim that the membrane supposed to be involved in osmotic interchange is permeable only to water and not to substances in solution. In addition to the membranes of the respiratory organs there is also the lining epithelium of the alimentary tract which doubtless also acts as an excretory organ.

Intracellular Excretion.—Durham showed that when India ink is injected into the body cavity of the starfish the numerous amœboid cells of the cœlomic fluid act as phagocytes and ingest the ink particles. They then wander out into the cavities of the respiratory organs where they coalesce into large masses, and finally with their enclosed burden of granules are cast out through the membranes of the respiratory papillæ. This form of elimination is common to the starfish, for it is found that even in individuals which have not received any injections of foreign materials there is an abundant elimination of colored granular matters derived from normal metabolism, and which have come to be included within amœboid cells. These are cast off through the respiratory papillæ and appear in the mucous covering of the body.

Similarly, the Echinoids show a process of intracellular excretion. Wandering cells collect granules of yellow-brown material and then migrate to the oral branchiæ, where they become massed at the outer ends of the gills or deposit their granules just beneath the outer epithelium of the gill. The waste matters are then extruded through the epithelium of the gill.

Among the Holothurians a very similar process takes place. Here the intracellular process occurs in the wall of the so-called respiratory tree. The branched lumens of the respiratory tree show an unusual concentration of yellow-brown granules and similar granules are found also in wandering cells located in the wall of the respiratory tree. Such cells, taken at the instant of elimination, form remarkable projections on the tree epithelium and are easily found in the water forced out from the respiratory tree during its rhythmic contractions. This form of activity was first demonstrated by Schultz, who saw amœbocytes of the cœlomic fluid containing India ink collect at the peritoneal surfaces of respiratory tree chambers, thence collect under the epithelium, fuse into large plasmodial masses filled with ink and excretion crystals, which then wander through the epithelium, finally to emerge into the lumen of the respiratory tree. This process goes on with considerable rapidity. In one case all the ink injected had been eliminated within 10 hours. While the process described is one artificially induced, it seems to follow the course of a perfectly normal process.

Intracellular excretion also takes place through the genital organs and their ducts. This is especially noticeable during the breeding season, when it is possible to find in ovaries and testes of the sea urchin cells which contain large masses of pigmented matter (presumably of an excretory nature). These granules come into the lumen of the organ from the cœlom, passing through the epithelium forming the wall of the duct. It appears that these granules are enclosed in amœbocytes, and that it is through the activity of these amœboid cells that the excretion granules are moved about from place to place.

Very little trustworthy information exists concerning the chemical nature of the substances excreted by the Echinoderms. The statements of different investigators are sufficiently contradictory to make it seem probable that they were not all dealing with the same substances. For example, Durham was convinced that the mucus from the end of the starfish arm contains no urea or urates. Schultz came to the same conclusion in regard to the crystals eliminated by the respiratory tree of the Holothurian. On the other hand, Bordas found evidence that in the terminal ampullæ and outer passages of the respiratory tree there are crystalline deposits which give reactions characteristic of urea and urates. Such depositions must have occurred as the result of diffusion of dissolved substances through the respiratory epithelium, and the subsequent crystallization of this material.

Storage Excretion.—Among the sea urchins and the Holothurians not all of the excretory matter diffuses through the superficial epithelium or through the walls of the respiratory organs. There is a definite accumulation of excretory material in the body. Granules and crystals of a brownish-yellow color occur either within the cells or lie free in the intercellular spaces of the body tissues. They are especially abundant in the subcutaneous connective and lacunar tissues, mesenteries, water-vascular system (particularly the Polian vesicles), and axial organ. The axial organ has been referred to by various authors as a heart, kidney, special gland (ovoid gland), and lymph gland. The last suggestion may be the best. Kowalewsky observed that when carmine is injected into the cœlom of Echinus or Strongylocentrotus the ovoid gland becomes thickly filled with the injected substance. It might be suspected that the organ is excretory in character, but for the fact that it has no duct, unless the idea is accepted that it may serve as a focus of excretion storage. This may be true in the Echinoids but is less likely to be true among the Asteroids.

The excretion granules and crystals are not formed in the places where they are found to be stored. They are transported to these localities by means of certain specialized amœboid cells known as *nephrocytes* or *athrocytes*. These cells have the power to collect soluble wastes from the cœlomic fluid or from the fluid in the water-vascular system. These wastes are carried as liquid in vacuoles or as crystals or granules within the cytoplasm to some point of intracellular excretion or to some storage point. Certain of the nephrocytes or athrocytes appear to have a tendency to collect specific substances. Thus, there are certain athrocytes which, under experimental conditions, collect carmine grains, others which collect indigo, etc. Some stationary cells, found in various parts of the body, have a similar power to absorb or ingest and store foreign matters. The existence of such cells in the walls of the alimentary tract in all classes of Echinoderms makes plausible the suggestion

that the alimentary tract or some part of it may serve as an excretory organ.

The accumulations of crystals and granules vary greatly in size, some being very small, others measuring several millimeters in diameter (the size of a pea).

The chemical nature of the deposits has not been determined, although numerous studies have been made. They do not respond to tests for guanin or xanthin and are insoluble in cold acids and in cold and hot alkalies.

Excretion among Worms.—Most worms possess some form of excretory apparatus. Exceptions to this general rule occur among the Turbellarians, Nematodes, and a few of the Polychæts and Oligochæts. As a class, the worms exhibit remarkable variations in form, size, and in the degree to which organic differentiation has taken place. In no system of organs is this variability more emphasized than in the excretory organs. Any account which may be given in a work of this kind is of necessity far from adequate. Attention, however, will be called to certain conditions which are, in a way, characteristic of the group.

An excretory system is fundamentally a structure designed for the elimination of wastes and for regulating the water content and the salt content of the body. These activities may involve elimination and a subsequent selective resorption of materials which have passed through the eliminative structure, so that the material finally passed off from the body has quite a different composition and concentration from that which originally appeared in the tubules of the excretory system. Among the freshwater protozoa, the eliminating system has become well developed, but apparently no provision is made for the resorption of dissolved materials which might be of value to the animal.

Among the worms and higher forms a system is developed which provides not alone for the elimination of liquid wastes, but there is associated with it a mechanism for the resorption of water, and possibly a mechanism for adding to the liquid certain waste matters as it passes outward through the tubules.

All organs having an excretory function possess an epithelium having its proximal face directed toward a body fluid containing wastes, and its distal face directed toward some conducting, resorbing, or retaining space.

This epithelium, which may be uni- or multicellular, is primarily a filtering or diffusing mechanism. No filter or diffusion membrane can work to advantage unless the pressure of liquid or of some constituent of it is greater on one side of the membrane than on the other. This difference of pressure may be hydrostatic—the pressure due to the contraction of muscle in the walls of blood vessels—or it may be osmotic—the pressure due to differences in the concentration of dissolved molecules on the two sides of the membrane. In any case there is the tendency

for diffusion or filtration to occur from regions of higher to lower pressure or concentration. Also, associated with the purely physical filtration or diffusion process there may be a definite secretory process involving the expenditure of energy upon the part of the protoplasm of the cell.

There is little evidence of outward selective diffusion through the membrane—except in the fact that certain substances such as albumin and sugar do not normally pass through the membrane in any considerable amounts.

The particular form which is assumed by the filtering mechanism varies from group to group of animals, depending to some extent upon the character of the liquid bathing the proximal surface of the membrane, whether it be hydrolymph, lymph, or blood from which the wastes are to be eliminated; varying also because of the differing demands made upon the apparatus in different animals, in some being merely the elimination of liquid wastes, in others involving the elimination of some insoluble matters as well. In some forms the organs are single cells; in others they are complex multicellular structures. Great variations occur also in the degree of elaboration of the glandular (secretory, as opposed to filtration) mechanism and activity of the organ and the provision made for resorption. From the simple solenocyte cell of the worm to the highly complicated tubular kidney structure of the mammal may seem to be a long jump, but in reality the two are much alike and apparently work in the same fundamental way.

Attention should be directed to an apparent *correlation existing between different types of excretory systems and circulatory fluids and systems,* as shown in the following table:

Collecting Fluids	Circulatory Apparatus	Excretory Apparatus
Forms having an intercellular hydrolymph which washes freely through the body parenchyma.	None	Tubular or sac-like organs. Tubules often much branched and completely closed at inner end. Terminal cells (flame cells) at ends of tubes act as secreting cells. Canals and collecting tubes intra- or intercellular.
Cœlomic fluid	Cœlomic fluid kept in motion by body movements. Fluid of vascular system propelled by contractile vessels.	Unbranched nephridia, sometimes segmentally arranged, which may be closed or provided with open nephrostome funnels. Tubules variously curved, and composed in part at least of glandular cells.

Collecting Fluids	Circulatory Apparatus	Excretory Apparatus
Blood	Elaborate systems of arteries, capillaries, and veins, filled with the rapidly circulating fluid. Intercellular and cœlomic fluids reduced to a minimum.	In higher forms terminal plexuses of capillaries press into closed ends of nephridial tubules = Glomeruli or Malpighian corpuscles. Nephridial tubules provided with capillary system. In higher vertebrates kidney is specialized out of nephridia, and designed to collect and remove urea and urates from the blood. Tubules serve in part as resorptive organs.

Arrangement of Excretory Organs among the Worms.—Among the lower worms the tubules of the excretory apparatus ramify throughout the whole of the body parenchyma (see Fig. 79), and bring all parts of the body in rather close relations with the terminal cells through which filtration takes place. Such an arrangement is necessary because of the inadequate means provided for keeping the body liquid in movement.

Fig. 79. Excretory canal system of a flatworm.

Among the segmented worms the excretory organs are segmentally arranged. Sometimes there is one pair of nephridia to a segment, sometimes more. Each segment of the worm may be thought of as a unit so far as the excretory function is concerned. It collects its own wastes and carries them to the outside of the body. In this connection it should be kept in mind that in such forms as the earthworm the funnel of the nephridium is in the segment anterior to that in which the major portion of the tubule lies.

Types of Filtering Mechanisms.—In a previous paragraph it was mentioned that rapid diffusion or filtration is facilitated by a difference of pressure upon the two sides of the epithelial membrane. Other physical factors than pressure may also be involved in this process. The epithelium which is the responsible agent in the filtration process may assume one of a considerable variety of forms, depending upon the animal group in which it is found. It may be unicellular, as in the flatworms, or multicellular, as in the segmented worms and animals higher in the animal scale. Among the flatworms the excretory system consists of fine branching tubules each of which ends in a *terminal cell*. There is considerable variation in the extent to which these terminal cells are

developed. Figure 80 represents the structure of a typical form. It will be noted that this cell is apparently a hollow, conical, or pyramidal structure, having its broad basal end (sometimes provided with branching processes) lying free in the hydrolymph which washes all through the body parenchyma. This broad basal end of the tubule really represents the membrane through which diffusion takes place. The surface of the cell exposed to the cœlomic fluid represents the proximal surface of the membrane. The so-called *flame chamber* of the cell really represents the distal surface of the membrane and not a cavity within a cell. The cilia which occur in the chamber are cilia on the distal surface of the membrane, or excreting cell, and their function is to propel from the surface of the cell, as rapidly as it appears, any liquid which may diffuse through the walls of the cell.

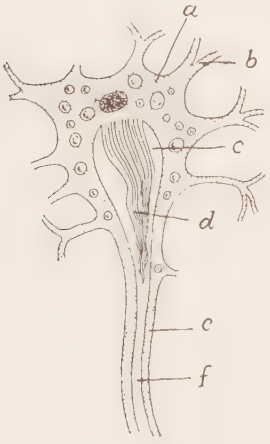


FIG. 80.—Flame cell. *a*, proximal surface of cell, exposed to body fluid; *b*, cell processes; *c*, flame chamber, formed by invaginated distal surface of cell; *d*, cilia forming "flame"; *e*, wall of tubule; *f*, lumen.

Types of Arrangement of Excretory Surface. It is but natural to expect that with increased complexity of animal structures, in passing in review from the lower to higher forms, there should be noticed a marked variation in the character of the excreting membrane, that membrane which lies between the circulating fluid of the body and the outer medium. Some of the conditions found are shown very diagrammatically in Fig. 81. The term *proximal* refers to the surface of the membrane exposed to the body fluid. The term *distal* refers to the surface exposed to the outer medium. In *A* there is represented the condition existing in any animal in which the general body surface serves as the membrane of elimination. In *B* there is shown the condition characteristic of the Echinoderms. Here, it should be pointed out, is a case in which there is no real separation between the functions of excretion and respiration. The so-called respiratory papillæ, pushed from the body surface into the sea water, serve as well for the elimination of wastes as for gas exchange. In *C* there is shown the condition existing among the insects, in which diverticula from the intestine, in the form of elongated, simple tubes, serve as organs of elimination, passing the matters to be excreted on to the digestive tract for final elimination. In *D* there is shown the condition found in the lower worms, where the so-called water-vascular system carries off from the body such substances as are able to diffuse through the flame cells into the tubule. In *E* there is shown a modification of flame-cell type of structure, in which a number of flagellated solenocyte cells allow water to diffuse through them into a common chamber and

so to be eliminated. In *F* there is shown a condition found among some of the Annelids, in which the nephridial tube, provided at its cœlomic end with flagella, is open to the cœlomic fluid. Here the liquid passes without hindrance out through the tubule. In *G* is shown a further development of the open tubule. The open end of the tubule in the cœlom has become expanded into a large ciliated funnel which serves as an admirable collecting organ not only for liquid, but for minute detritus. In this type of nephridium the tubule may become very much elongated and glandular (a feature not shown in the diagram). In *H* there is illustrated a sort of transitional stage between that of the open-funneled condition and that existing in the kidney of the higher animals. The nephrostome is not only open to the cœlomic fluid, and provided

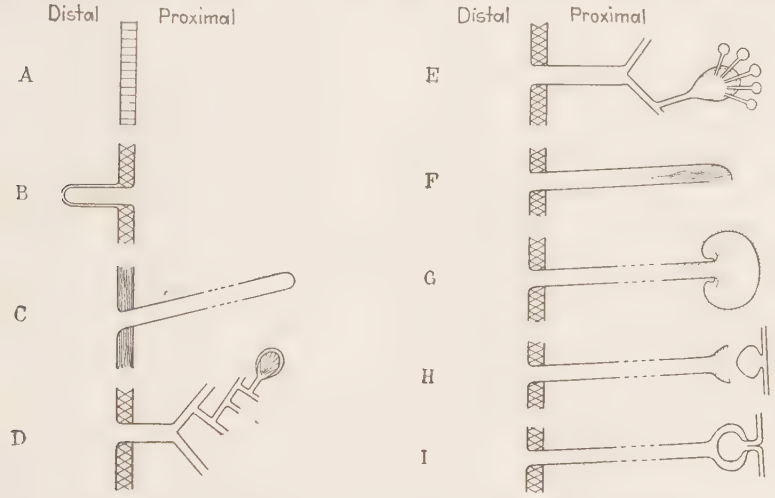


FIG. 81.—Types of excretory surface (for description see text).

with cilia so as to draw in cœlomic fluid, but there is in addition a glomerulus of the blood-vascular system brought into close relations with the open funnel. Such an arrangement provides for diffusion of liquid from the vascular stream and the taking up of that fluid by the nephridial tube. In *I* there is illustrated the condition existing in all of the higher animals, in which each nephridial tube has its proximal surface expanded into a flask-like chamber and then invaginated so as to form a large inner space surrounded by two thin walls. Into this large inner space there is inserted a glomerulus or tuft of capillaries. From these capillaries water and dissolved salts, etc., are able to diffuse into the cavity of the capsule, which leads directly into the tubule of the nephridial tube. Here again the character of the tubule is not indicated in the sketch. The wall of the tubule is, for a great part of its length, composed of glandular cells, whose special work is to resorb from the tubule water

and such substances contained in the water as may be needed by the organism.

In some cases there is a combination of two of these elementary types in the make-up of a kidney structure. For example, the kidney of the frog contains structures of the *F* and *I* forms. In this case the *F* portion empties not into the *F* tubule, but into one of the veins which permeate the structure of the kidney. In some other forms both tubules have a common outlet.

A very interesting modification of the flame cell structure is found in the so-called solenocyte. Here the excretory tubule ends in a closed cavity or terminal chamber, through the end wall of which project tube-like extensions of several cells, each of which is provided with a single large flagellum which reaches down through the length of the tube and vibrates freely in the liquid contained within the chamber (see Fig. 82*C*).

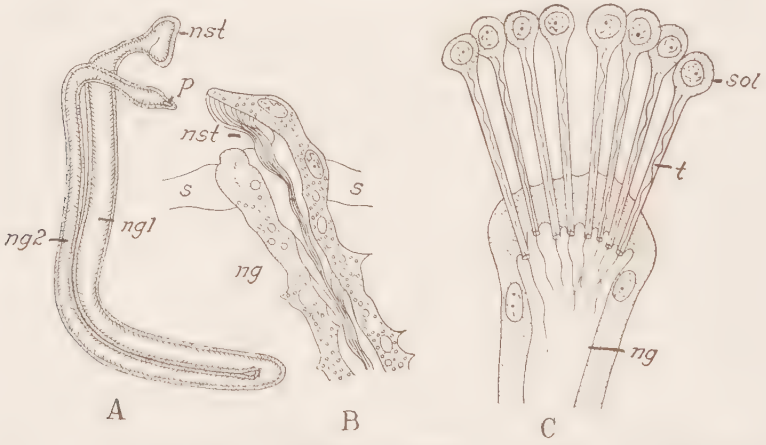


Fig. 82.—*A*. Nephridium of *Scalibregma inflatum*. *B*. Section through nephrostome of *Trypanosyllis*. *C*. Solenocyte apparatus of *Phyllodoce Paretti*. *ng*, nephridial canal; *nst*, nephrostome; *p*, nephridiopore; *s*, septum; *sol*, solenocyte cell; *t*, solenocyte tube.

In some cases the solenocyte cells are exceedingly numerous. Here the lashing of the flagella in the tubes and in the chamber tends to propel any contained liquid toward the distal end of the tube and thus lowers the pressure in the terminal chamber and solenocyte tubes. This lowering of pressure makes it possible for more liquid to diffuse through the cells into the tubes and into the chamber.

Among the higher worms, as, for example, in the earthworm, the nephridial tube opens at its inner end through a ciliated funnel, the nephrostome, into the cœlomic cavity. There is a great deal of variation in the structure of the nephrostomes in different forms (Fig. 82*A*, *B*), some being simply the open ends of the nephridial tubes provided with flagella; others are quite complicated structures, as in the earthworm Lumbricus. In these forms in which are found the open-ended tubules, filtra-

tion and diffusion are not the factors depended upon for filling the tubule with liquid. The water currents set up and maintained by the flagella or cilia cause the flow of water in the tubes.

As the nephridial tubes of different forms are examined there arises the question as to why so long a tubule as is frequently found should be developed between the nephrostome or flame chamber and the outer opening of the tubule upon the body surface. If the function of the nephridial tube is simply to reduce the amount of water in the body, a nephridium provided with a short tube between the part which takes up the water and that which gives it off would suffice. If, however, the

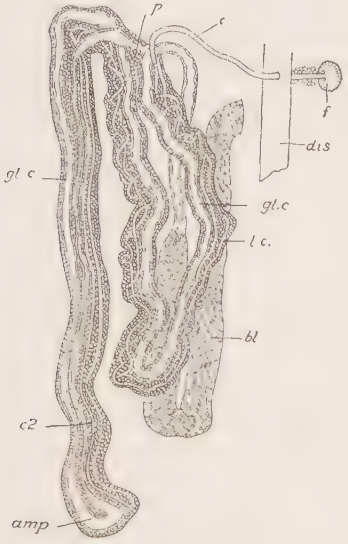


FIG. 83.

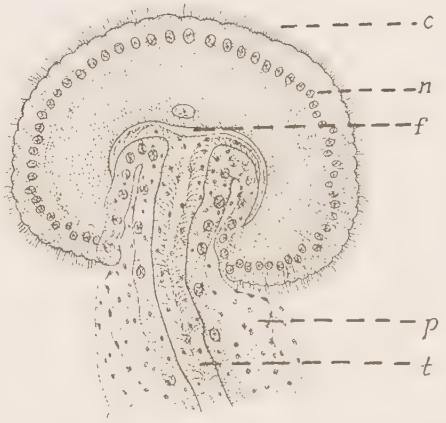


FIG. 84.

FIG. 83.—Nephridium of *Lumbricus* sp. *c*, first ciliated canal; *f*, funnel; *dis*, dissepiment; *gl.c.*, glandular canal; *l.c.*, loop canal; *p*, passage of loop canal into second ciliated canal; c^2, second ciliated canal; *amp.*, ampulla; *bl*, urinary bladder. (*Ac. Benham, from Schneider.*)

FIG. 84.—Funnel of nephridium of *Lumbricus*. *c*, ciliated cells of funnel; *n*, nucleus; *f*, funnel; *p*, peritoneum; *t*, tubule.

tubule itself plays some part in the work of excretion, it might well need to be quite elongated.

The nephridium of the earthworm is one in which the tubule becomes greatly lengthened. The form of the nephridium of the earthworm is well shown in Fig. 83. While in some of the lower forms the nephridium consists of a nephrostome and neck, a middle piece, and an excretory pore, in the earthworm it becomes a much more complicated organ. There is here a nephrostome (Fig. 84) which leads into a narrow, thin-walled, looped canal; then a thicker-walled, ciliated loop. The ciliated cells of the thicker-walled loop are filled with yellowish or brownish granules. This section of the tube ends in an ampulla. Out from the ampulla

leads a glandular tube which leads into the so-called *rod canal*. The name rod canal comes from the presence of rod-like structures in the bases of the cells forming this portion of the tubule. This section of the tubule finally empties into a broad bladder-like terminal portion. The constriction of the muscle fibers forming the wall of the bladder portion shuts off the end of the rod canal and so prevents the liquid from passing backward along the tubule.

With regard to the function there are three possibilities:

1. It may serve simply as a conducting tube.

2. The cells of the tubule may remove something from the filtrate as it passes down the tube.

3. The cells of the tubule may add something to the filtrate.

The fact that the cells of the tubules are histologically very much like the cells composing the wall of the kidney tubules of the vertebrates indicates the possibility that there may be a similarity of function. It is well known that the tubules of the vertebrate kidney have a glandular activity.

Rate of Excretion.—The rate of streaming of the nephridial content and of the emptying of the end bladder varies widely in different forms. The pressure necessary to keep the fluid in motion comes from the activity of cilia or flagella, or, in the leeches, from the contractions of an inner circular muscle sheath. The amount of liquid passed through the nephridia of the different forms varies greatly. In some of the Rotifera an amount of water equal to the weight of the body is passed through the nephridia in the course of a few minutes, while the movement of the liquid in the Annelids is incomparably slower. In fact, the period of retention of the urine is so long, in some cases, that some observers have concluded that the nephridial pores are definitely blocked. Cuenot, however, concluded that the nephridial content is voided at least once in 3 days. The very great length of the nephridial tubules in the Annelids and their very fine bore bring about a very high capillary resistance, and the streaming comes almost to a standstill—and undoubtedly would cease were it not for the ciliary activity within the tubule.

Functions of Different Parts of Nephridia.—It is very generally conceded that the terminal cells, flame cells, solenocytes, etc., are the structures primarily concerned in the elimination of water, in the forms in which they occur. In the forms in which the proximal ends of the nephridial tubes are open to the cœlomic fluid there is no question as to how water, dissolved salts, and even granules may enter the tubule.

There are found in the terminal cells, flame cells, etc., colorless liquid drops or vacuoles, and variously colored granules similar to those which appear in the content of the tubule. Among the lower worms the cells forming the walls of the tubules are thin and give little indication of any other than a conducting function, so they may be eliminated as possible

manufactories for excretory substances. Variously colored, yellow, brown, or black granules, similar to those found in the nephridia, occur in the cells of the body parenchyma as well as in the terminal cells; hence it may appear that the granules may be passed on directly from the parenchyma cells to the terminal cells, possibly through the cell processes of the latter, and then be eliminated. Such a passing of granules from cell to cell is not at all uncommon among the lower forms. Or it is possible that the granules may be manufactured in the terminal cells from the liquid material which passes through them, and these may then be passed on through the tubules for final elimination.

Among the Annelids there is a somewhat more complicated condition. The liquid entering the proximal end of the tubule, either through the solenocytes or the open funnel, is water containing substances in solution. Solid particles may enter the open funnel in the cœlomic fluid, but there is little, if any, evidence that the solid excretory granules are formed in solenocyte cells. The body fluid of some worms contains solid protein matter of value to the animal (Cuenot). Such material runs the chance of being lost unless the cells of the tubules may have the power of resorbing material as it passes by them. There is evidence that some of the cells of the tubules act as phagocytes and so may pick up and save for the organism solid matters of value. The ability of the tubule cells to take up solid particles does not also indicate ability to build up solid particles out of dissolved materials. Botazzi has demonstrated by the freezing-point method the probability of the absence of albumen and other soluble proteins from the fluid contained within the tubules, which in marine forms has the same freezing point as the sea water in which the animal lives.

The solenocytes and the cells of the nephrostomes always lack colored enclosures and probably play no part in the handling or elaborating of excretory matters. The glandular portions of the nephridial tubes, whether open to the cœlomic fluid or not, however, have their cells regularly filled with solid and liquid enclosures. There appears to be no doubt in the minds of those who have made the investigations that these enclosures, vacuoles, granules, etc., are excretory material, and that these substances are finally pushed out from the cells into the lumen of the tubules, and from these eventually eliminated. Two methods by which excretory substances may be thrown off from the walls of the nephridial tubes have been suggested: (1) *merocrine* excretion, in which the gland cells give off to the lumen only the secreted substance (Fig. 85*A*), and (2) *holocrine* excretion, in which the secretion is freed by the breakdown of the whole protoplasm of the cell in which it lies (Fig. 85*B*). Of these, the first is the more general. In either case the materials are carried into the vehicle stream and soon pass to the exterior.

There seems to be no doubt in the minds of those who have studied excretion among the worms that the cell enclosures mentioned above are formed from material which is in solution in the circulating fluid of the body. The amounts of these substances in the cœlomic fluid are so small that they cannot be detected by ordinary chemical analyses.

The cells of the nephridial tubules which do the work of collecting and concentrating the excretory materials have an activity similar to that of the *athrocytes* of the Echinoderms. They are cells which have the power to take in from the cœlomic fluid waste matters, solid or liquid, and to deposit them within themselves as drops or granules. Also there are wandering, phagocytic cells which pick up wastes from the cœlomic fluid and carry them to the tubule cells which later excrete them.

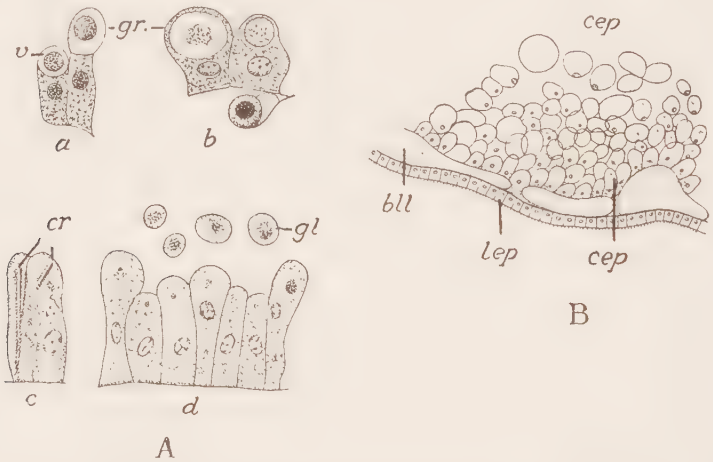


FIG. 85.—*A*. Merocrine excretion precursors in cells of cœlomic sac. *a*, *Galathea; b*, *c*, *d*, lobster; *v*, vacuole; *gr*., spherical granular mass; *gl*, free excreted ball; *cr*, crystals. *B*. Holocrine excretion precursors in cœlomic sac of Calappa granulata. *cep*, epithelium of cœlomic sac; *lep*, epithelium of labyrinth; *bll*, blood lacuna.

Excretophores.—In addition to the nephridial tubes there are other structures in the Annelids which have the power to take up dissolved wastes and to collect them into semiliquid or solid masses. Such structures are known as excretophores. While these structures vary much in form and in location in different worms, they have the following characters in common:

1. They originate from the cœlomic epithelium.
2. They build up the material of their enclosures from substances circulating in the blood (cœlomic fluid).
3. They sooner or later are cast off into the cœlom.

Sometimes the excretophores are found within the walls of the blood vessels, in which case they are spoken of as *intravasal excretophores;* sometimes they are outside the blood vessels, in which case they are

called *extravasal excretophores*. There are many forms among the Annelids which possess intravasal excretophores, in the form of bands or strings of cells in the dorsal blood vessel or *heart*, and which are commonly known as heart bodies. These heart bodies may be specialized glands (sometimes referred to as hæmoglobin factories) or they may play a part as mechanical valves for directing the course of the blood stream. The fact that these bodies have also an excretory function is shown by the wealth of their granular enclosures, and by the fact that the bodies are neither protein, carbohydrate, nor fat. Also the cells composing the excretophores have, in general, the character of athrocytes. The concrements formed in the cells of the excretophore do not long remain there, but soon escape to the blood stream, in which they are carried to the nephridia. Here they are eliminated.

Extravasal excretophores occur fully as frequently as the intravasal among the Polychæts, and among the Oligochæts they are most common. They take the form of more or less hypertrophied peritoneal cells which cover the outer walls of certain of the larger blood vessels, and at times are so massed as to give the appearance of being special organs. They are filled with strongly refracting, yellowish or darkly colored granules. Also spreading from the blood vessels they may come to surround not only the dorsal blood vessel, but also the whole of the stomach intestine, as is done by the chlorogogue cells of the earthworm. Even here the inner ends of the cells come into close relations with the blood vessels (see Fig. 86). The chlorogogue tissue was for a long time thought to be involved in the production of digestive enzymes, but this idea was dropped when it was seen that there is no way for such a secretion to be poured into the intestinal lumen. Cuenot looked upon the chlorogogue tissue as one for the temporary storage of protein. Timm, Kukenthal, Grobben, Kowalewsky, and others of later date hold that the chlorogogue is an excretophore tissue and that the granules seen in the cells are excretory material. The chlorogogue cells are easily broken loose

FIG. 86.—Piece of longitudinal section through wall of stomach intestine of *Lumbricus*, showing two groups of chlorogogue cells. *st.*, stalks of chlorogogue cells; *lm.*, longitudinal muscle fiber; *cm.*, circular muscle fiber.

from their attachments and may be carried about by the currents of the cœlomic fluid. Later, they may again become attached and reestablished as fixed cells. The cells are large, have a clear protoplasm and contain vacuoles and granules of varying sizes, colors, and numbers.

The botryoidal (like a cluster of grapes) tissues of certain of the worms and the highly modified peritoneal cells covering the mesenteries and the fore and hind guts among the Gephyrean worms are evidently of the same general sort and serve as excretory structures.

The chemical nature of the substances contained within the excretophore cells is similar to that of the bodies found within the nephridial cells. The yellow granules are largely guanin, while the kidney-shaped bodies and membrane-like inclusions are chitin. In the leeches and Sipunculids the botryoidal tissues have been shown to contain urates. While the chemical nature of the substances thrown off by the excretophore cells has not been fully investigated, it is stated that it is, in general, essentially the same as that of the bodies which appear in the cells of the nephridia of the same animal.

Fate of Excretophore Substances.—Sooner or later the enclosures of the excretophores reach the body cavity. This is accomplished by: (1) the disintegration of the excretophore cells and the scattering of their contents in the cœlomic fluid, or (2) by throwing off the outer ends of the excretophore cells and so scattering the enclosed granules, or (3) by dislodging the cells which for a time lead a separate existence in the cœlomic fluid.

In either of the two latter cases the cells soon suffer a cytolysis and the enclosed granules become scattered, sometimes being noticeably altered in appearance, sometimes even being broken up into fine detritus. At other times the destruction of the granules is not completed until they have been removed from the cœlomic fluid. The phagocytic corpuscles of the cœlomic fluid serve as a means of transport. They ingest the particles found in the cœlom, whether they be cells, cell fragments, or granules, and carry them to some point of elimination or storage.

The amœboid cells of the cœlomic fluid are highly phagocytic. Any foreign particles introduced into the body cavity of one of the higher free-living worms are quickly taken up by the blood cells. India ink, carmine grains, etc., as well as particles formed within the organism, quickly become stuck to the surface of the leucocytes, and then are drawn into the ectoplasm, then into the endoplasm of the cells. Large particles, too large for a single corpuscle, will be attacked by numerous cells and finally be encapsuled by a slimy mass. At times the amœboid cells take up matter which had been formed within chlorogogue cells, but had been cast into the cœlomic fluid. These amœboid cells, charged with excretory substance, come to be wandering cells.

There are also lymph cells sticking firmly to the cœlomic wall which have phagocytic powers. Such cells, after having taken up particles from the cœlomic fluid, may become free and pass through a history similar to that of ordinary lymph cells. One of two possible destinies confronts these wandering cells and their excretophore content.

1. In many of the worms these wandering phagocytes and lymphocytes become aggregated upon the ciliated funnel sacs, there to undergo, first, an agglutination, then digestion and dissolution, and, finally, elimination through the nephridium.

2. The cells may carry the granules to some place of excretion storage and there become changed into immobile cells, or become dissolved and leave their contents enmeshed between the tissue cells. There are great variations among the worms in the degree to which excretion storage is developed. In some cases it only temporary, while in others it is a permanent affair. The tissues chiefly involved are muscle, epidermis, and cuticle. In many worms the pigmentation of the skin, particularly of older individuals, is an indicator of the extent to which this type of excretion has occurred. Frequently among the segmented worms there are found large masses of excretory material stored in the posterior segments of the body.

In some cases where the phagocytes or athrocytes clump upon the nephridial funnel, it is possible for the detritus arising from the cellular disintegration to pass directly into the nephridial tubule. There are many cases, however, in which the openings from the cœlom into the nephridia are so small as to hold back all but the very smallest particles, those having a diameter of more than from 5 to 8 microns being too large to pass. Occasionally, it happens that a nephrostome will open widely to allow chlorogogue granules and uric acid crystals to pass.

In those cases in which the nephridia do not open toward the cœlom some other method of disposing of the excretophore granules must be found. The granules appear to become dissolved in the cœlomic fluid about the nephridia or in the funnel sacs, and their substance to be resorbed by the cells of the nephridial tube, from which it is again eliminated in the form of drops or granules. Another method of disposal occurs in some forms: Phagocytes filled with excretophore granules at first adhere to the nephridial cells, then fuse with them, so that the enclosures of the free cells become the enclosures of the nephridial cells. From the nephridial cells these enclosures are passed on to the lumen of the tubule, and are then eliminated through the nephridiopore. In some of the Lumbricidæ it has been shown that this second method, involving the fusion of cells containing excretophore granules with nephridial cells, takes place even in nephridia which are open to the cœlom. Some authors have felt that the condition existing is rather one of diapedesis, *i.e.*, of an amœboid cell forcing its way through a membrane and then

of an intracellular excretion similar to that which has already been described for the Echinoderms. Such an intracellular excretion taking place in the nephridium is quite rare. On the contrary, however, intracellular excretion is quite common at the surface of the body and occurs to some extent from the wall of the alimentary canal. This last type of excretion is quite closely related to the storage excretion mentioned above, and it is quite possible for one to be mistaken for the other.

The processes of excretion among the worms have been considered in some detail since in this group there is a rather wide range of activities resulting in the elimination of wastes, but also illustrating the general principles of excretion to be found among the higher animals, together with some indication of the evolution of the higher kidney type of excretory organ out of the simpler nephridial forms. Attention has been directed to an apparent correlation between the character of the body fluid and the character of the excretory apparatus.

In so far as the author is aware, no investigator has called attention to another seeming correlation, that existing between the character of the environment and the form of the nephridial tubule. No mention has been found of the fact that, in general, the aquatic worms have nephridia with short tubes or sacs, while the forms living in the earth have much longer tubes which are frequently much coiled. In the accounts of excretion there is found mention of the fact that the gland cells of the tubules may remove something from the blood or cœlomic fluid and add it to the liquid contained within the lumen of the tubule, but the writer has failed to find any mention of the possibility that the nephridial tube may also serve as a means of conserving water which might otherwise be lost to the organism. Animals which live constantly in the water would have little use for a water-conserving mechanism. The existence of such a contrivance becomes important in proportion to the possible dehydrating influence of environmental conditions. If the short nephridial tube and sac of such a form as Nereis, for example, can carry off the wastes produced by this animal, it would seem plausible that a nephridium of similar structure could do the same thing for such a worm as Lumbricus. In the latter form, however, the glandular part of the nephridial tube is relatively much more highly developed. This extreme development must occur for the purpose of meeting some need on the part of the earthworm that does not equally occur in the marine worm. Such a need would be that of conserving the water content of the body. There are hints in the literature that the urine becomes more concentrated as it passes down along the tubule. It has certainly been inferred that this concentration is due to the addition to the liquid in the tubule of solids excreted by the gland cells. It seems appropriate to raise the question whether such concentration may not as well be accounted for upon the supposition that water is resorbed from the nephridial tube and saved

to the cœlomic fluid. Further investigation is needed at this point. If this suggestion shall be found of value, it will make apparent a very close harmony between the workings of the worm's nephridium and the tubule of the mammalian kidney.

Excretion among Molluscs.—Definite excretory organs occur almost universally among the Molluscs. In their fundamental structure they bear strong likeness to the nephridia of the worms. The fact that the body of the Mollusc is unsegmented has resulted in some modification of the structure, as will be pointed out presently. These excretory organs are lined with cœlomic epithelium, and are bent more or less into an S form, opening at the proximal end into the cœlom through a nephrostome. The cœlom in these forms is very decidedly reduced, the chief part of it being the pericardial cavity. The distal end of the excretory organ opens at some point upon the surface of the body through a nephridipore. The wall of one portion of the tubule is made up of glandular cells which come to be very richly filled with granules and concretions.

In certain respects the excretory organs of the Molluscs contrast rather strongly with those of the worms. While the number of nephridia to be found in a worm varies with the size and the number of segments making up the animal, there are to be found among the Molluscs only a very few of such structures. Typically, a single pair of symmetrically arranged nephridia occur in a great many forms. Among the Opisthobranchs and Pulmonates and a few other forms there is only a single nephridium to be found. Among some of the Cephalopods there are two pairs. This reduction in the number of excreting structures is evidently a direct response to the increased efficiency of the circulatory apparatus. The blood circulates so rapidly, along definite courses, that widely distributed eliminative structures are not needed. The same principle holds for all the higher animals. And it seems logical that these structures should be closely associated with the heart, in view of the fact that the cœlom is thus reduced to a pericardial cavity, and that cœlomic epithelium is the tissue chiefly involved in the matter of excretion. Among a few of the Molluscs, water taken into the nephridial tube from the outside through the nephridiopore serves as the vehicle for carrying off the wastes. In most forms some special provision is made for the elimination of water. The cœlomic epithelium lining the pericardium becomes specialized into a pericardial gland, the excretion from which is decidedly acid. Such glands occur on the surfaces of the auricles or in ramifications of the pericardial sac among Gasteropods, Lamellibranchs, and Cephalopods. The glandular wall is well supplied with blood vessels and in a way plays a rôle like that of the Malpighian corpuscle of the kidney of the higher animals.

The neck portion of the nephridium becomes elongated into a nephropericardial canal. The glandular portion may become broadened into a

sac-like structure. In some cases the walls of the sac become a much folded or even spongy tissue, with the result that the excretory surface is very greatly increased in amount. Among the Chitons the excretory organ becomes a much-branched canal system, the ramifications of which extend to all parts of the body.

It has seemed to some that the opening of the excretory pore into the mantle cavity constitutes a structural difference from the typical conditions existing elsewhere. It must be kept in mind that the excretory pore is really on the outside of the body even though the folds of the mantle and the shell may form protective coverings about the body mass. Any liquid thrown into the mantle cavity is just as truly outside of the body of the Mollusc as it ever can be.

The process of excretion apparently takes place about as follows: Water containing hippuric acid and possibly some other nitrogen compounds diffuses through the epithelium of the pericardial glands (Keber's organ) into the cœlomic space. Even where the pericardial glands are not well developed, it still appears that the pericardial epithelium plays an important part in the elimination of metabolic by-products. From the pericardial chamber the liquid is passed through the ciliated funnel into the nephridial tube. The cilia of the funnel are evidently responsible for maintaining the necessary pressure gradient to insure continuous filtration into the cœlomic cavity. As the liquid passes down through the nephridial tube it become more concentrated. It has been customary to assign an active excretory function to the cells of the nephridial tube, and if conclusions are to be drawn from the appearances figured by various investigators, it would seem correct to suppose that there are added to the liquid as it passes down the nephridial tube contributions of granules and concretions. The possibility of water being resorbed from these tubules into the blood as it circulates through the small blood vessels of the nephridium should not be forgotten. The solids which are added to the liquid in the nephridial tube consist chiefly of uric acid and its salts. Sometimes lime and phosphoric acid are found. Among the mussels there is a sort of storage of these last-mentioned substances, underneath the epithelium of the nephridium, in the form of kidney stones. These may become as much as 3 or 4 millimeters in diameter. Except for the formation of these kidney stones the excretions of the nephridial cells are emptied into the lumen of the tube. This emptying process may be merocrine or holocrine, depending largely upon the quantity and quality of the cell enclosures. In addition to the inorganic salts eliminated, traces of creatin, creatinine, taurine, and some of the ferric salts have been found. Among the Cephalopods crystals of uric acid are eliminated, although repeated tests have failed to indicate the presence of uric acid in the liquid. These uric acid crystals may possibly have their origin in certain parasites which infest the animals.

In addition to the nephridia there occur among the Molluses, as among the worms, other methods of disposing of wastes. As an analogue to the excretophore tissues of the worms the Molluses have developed the pericardial gland, the major part of whose secretions are carried off through the nephridial tube. A portion of the secretion is, however, disposed of by amœboid phagocytic cells. As an example of glandular structures which collect and hold waste matters among the Molluses may be mentioned the *concrement glands* of Cyclostoma. These contain xanthin and hypoxanthin. In other forms certain of the mesenchyme cells of the body form concretions which are said to contain uric and hippuric acid crystals. Phagocytic cells, chemotactically directed, also take part in the excretory activity. They come to the cells charged with wastes, take into themselves masses of waste particles, and wander to the surface of the epithelium of gills, palps, etc., and there cast out their burden of wastes. This form of intracellular excretion has been noted especially in the mussels, the Prosobranchs, and the Cephalopods. Among the Pulmonates intracellular excretion does not occur, but there

FIG. 87.—Section through the kidney of *Helix pomatia*. *a*, urinary granules; *b*, epithelial cells; *c*, connective-tissue nucleus; *d*, connective-tissue septum.

are deposits of lime in the connective tissues. Also so-called excretory pigments appear in the skin and shell.

During *hibernation* there is a general lowering of activity upon the part of animals. The snail Helix fails, during the hibernating period, to throw off from the nephridia (Fig. 87) such wastes as may accumulate. This may continue for so long a time as 5 months. The individual cells of the tube, however, give off at a very slow rate such excretions as may be formed, and these gradually pile up in the tube. As soon as the animal becomes active the whole mass is passed to the outside. The mass of excreted material contains about the amount of uric acid that would, under normal conditions, be produced in about 3 weeks, thus giving an indication of a very much slowed metabolism.

Excretion among Crustacea.—While there are among the Crustacea several ways of disposing of waste matters, there are two sets of glands

among the representatives of the group to which special excretory functions have been assigned. These are the antennary glands, which empty at the bases of the second antennæ, and the shell glands, which empty at the bases of the second maxillæ. In some larval forms both sets of glands exist; in adults only one set persists. Each of these glands is considered as a modified segmental gland, similar in character to the segmental glands of the Annelid worms. Since the number of the glands is limited in every case to two, it is natural that these two should be well developed in order to meet the demands of the organism. And it is a matter worthy of mention that two such glands could hardly meet the needs of the organism without the aid of a very highly developed and efficient circu-

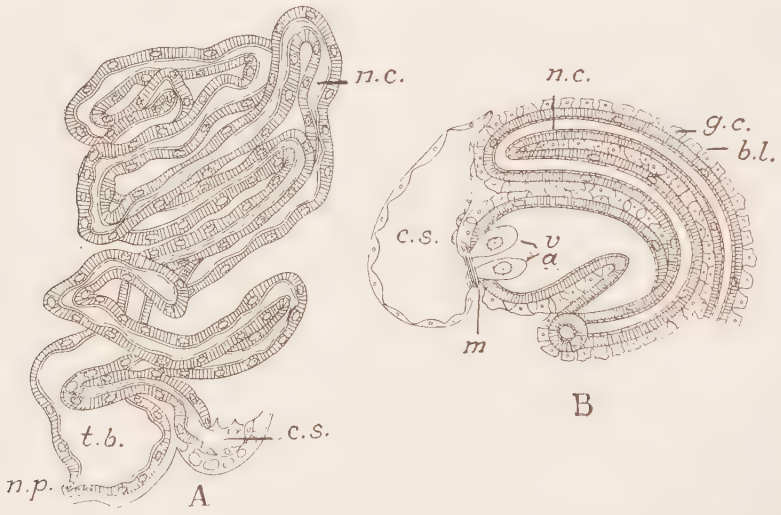


FIG. 88.—*A*. Antennal gland of *Mysis*. *B*. Cœlomic sac and the beginning of nephridial canal of *Gammarus*. *a*, ampulla; *c.s.*, cœlomic sac; *m*, sphincter muscle; *v*, valve; *n.c.*, nephridial canal; *g.c*, gland cells; *b.l.*, blood lacuna; *t.b.*, terminal bladder; *n.p.*, nephridial pore.

latory system. Each gland (Figs. 88 and 89) consists of a terminal sac lined with epithelium; a coiled, sometimes much elongated, urinary tubule, and a urinary bladder. The cells of the walls of the terminal sac are distinctly of the glandular type, and the walls are frequently much complicated by foldings. In some forms the urinary tubule is intracellular, the wall consisting of only a single cell; in others it is intercellular. The wall of the urinary bladder is in some of the higher forms also complicated by lateral invaginations.

The physiology of these glands has been subject to much study. Haeckel, Lemoine, and Huxley decided that they are excretory in nature, and based their conclusions, in part at least, upon the statement of Borup-Besanez and Will, who say that the gland gives off a secretion containing

guanin. Experiments made by injecting various colored granules into the body of a crayfish show that it is possible for the antennary glands (green glands) to eliminate such foreign particles. Kowalewsky decided that the end sac is the analogue of the Malpighian body and that the tubule is the analogue of the tubule of the vertebrate kidney.

In addition to the excretory work of these structures just mentioned it has been found that the cells of the so-called *liver* have the power to take up and to pass into the alimentary tract foreign particles which have been injected into the blood. Among the lower Crustaceans the lining epithelium of the intestine may also serve as an excretory organ. In some cases also, as among the Cirripedia, the skin serves as an excretory organ.

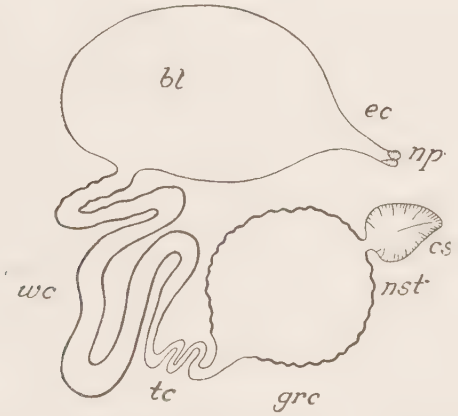


FIG. 89.—Diagram of antennal gland of crayfish. *cs.*, cœlomic sac; *nst*, nephrostome; *grc*, green canal; *tc*, transparent canal; *wc*, white canal; *bl*, urinary bladder; *ec*, terminal canal; *np*, nephropore.

Mode of Excretion.—P. Marchal made a very thorough study of the process of excretion among the Crustacea. He found in the liquid contained within the urinary bladder of the hermit crab Eupagurus, rather large, spherical, vesicular structures which disappear upon the addition of ammonia. These vesicles or bladders are formed on the inner ends of the epithelial cells of the end sacs and are given off to the liquid. The basal ends of the cells are quite opaque, striated, and filled with granules. When indigo carmine is injected into the body of a crab the colored material appears inside these excreting cells in the liquid contained within the vesicle. The crabs form very favorable objects for the study of the process of excretion. Here it is possible to pass a small catheter through the excretory pore, and thus draw off the liquid contained within the urinary bladder. From a single specimen weighing 50 grams there was obtained 13 cubic centimeters, and an hour later 6 cubic centi-

meters more. Another specimen gave 17 cubic centimeters. Normally the urine is emitted rhythmically, in some cases with considerable force, in others slowly. This emptying of the bladder is here apparently as important a function as it is in the higher animals, since animals whose excretory pores are mechanically stopped die in from 8 to 16 days.

Excretion by Fat Bodies.—Many insects show accumulations of uric acid in their fat bodies, and it sometimes happens that in mature insects the whole of the fat body becomes an accumulation of wastes, the fat cells being almost wholly replaced by the concrements. Among the Hymenoptera there is, immediately after metamorphosis, a great elimination of uric acid which had been accumulating during the larval period. This involves the going into solution of the uric acid so as to pass the Malpighian tubules in soluble form. Ceradot found that the Malpighian tubules of the rhinocerous beetle contain an abundance of uric acid, and that there are deposits of uric acid in the hind gut, but never anterior to the point of attachment of the tubules. Pasteur and Roulin found that the uric acid content of the tubules of the silkworm steadily increases in the interval between two molts, and that this disappears at once after the molt occurs, while there is an elimination of the accumulated uric acid to the outside at the molting time.

It seems certain also that in some cases the intestine serves as an organ for the elimination of uric acid. Krukenberg found in the beetle Osmodesma the intestinal lumen was filled for two-thirds of its length by a white mass of uric acid grains. This might be explained as a case of antiperistalsis, but the fact that in other insects, *e.g.*, wasps, there are unmistakable evidences of uric acid excretion by the intestine lends weight to Krukenberg's findings. Marchal is of the opinion that in some cases the intestine does the work which the Malpighian tubules, for one reason or another, are unable to do.

While uric acid is the most important metabolic end product among the insects, it is not universally so among Arthropods. It is doubtful if the Malpighian vessels of the Arachnids excrete uric acid. Here guanin is a more important metabolic product. This has been shown through the work of Gorup-Besanez, Davy, Plateau, and Weinland. The last-mentioned investigator found traces of uric acid as well as guanin. Calcium oxalate and calcium carbonate are also excreted by Arachnids.

Excretion among Insecta.—Among the great majority of forms the excretory organs are in the form of tubes or threads which occur in varying numbers as diverticula of the hind gut (Fig. 90). These are termed *Malpighian tubes* or vessels, and may possibly be modified segmental organs. The Malpighian vessels of the Arachnids are evidently analogous, though not homologous, to the Malpighian vessels of the insects, the former being of endodermal origin and the latter of ectodermal. Some authors deny that the Malpighian vessels of the Arachnids are

excretory in character, but there seems little real foundation for such a contention.

The Malpighian tubules usually arise at the junction of the mid- and hind guts. They are variable in structure and in number. The Diptera have two pairs; the Coleoptera, two or three pairs; the Lepidoptera, three pairs; and the Hymenoptera and Orthoptera, many short tubes. There appears to be a distinct inverse correlation between length of tube and the number of tubes to be found in any animal. Each tubule consists of three layers, a serous layer, a basement membrane which may be of connective-tissue origin, and a lining layer of secretory epithelium. The secreting cells are large in proportion to the size of the tubule, and frequently leave only a small lumen at the center. In some forms with very short Malpighian tubules there seems to be a completely closed lumen. The cells have, in some cases, deeply striated outer borders, and sometimes they bear cilia on their inner surfaces. The cytoplasm contains granules, crystals, and concrements of varying sizes and in varying numbers. It was the discovery that some of these particles are uric acid that gave the first information as to the real function of the tubules.

FIG. 90.—Boundary between mid- and hindgut of *Apion flavipes*, with modified Malpighian tubes.

Leydig discovered that there are two sorts of Malpighian tubules:

1. There are in the mole cricket whitish tubules which have their lumens filled with solid wastes. These give the white color to the tube.

2. There are also yellow tubules made up of secretory cells which contain granules, but there are no granules in the lumens of the tubes.

The suggestion that the Malpighian tubules are excretory in character was tested out experimentally by feeding various insects with food which had been poisoned. The cockroach Blatta and the beetle Cerambyx were fed on flour to which arsenic had been added, and the Orthopteron Mantis was fed upon flies which had been deprived of their wings and had then been sprinkled with arsenic. After 40 days of feeding the animals were examined chemically and arsenic was found localized only in the Malpighian tubules. The general health and activity of the insects were not interfered with in any way by the poisoning. Similar experiments upon the Myriapod Scolopendra gave the same result.

The elimination of stains has been studied by several investigators. The general results of these experiments may be summarized as follows:

Author	Form studied	Injection	Result
Schneider.....	Mole cricket	Sodium-indigo-sulphate	After 2 hours the outer parts of Malpighian tubules was stained. After 24 hours nuclei were stained blue, and the cytoplasm was clear. Later only the cytoplasm adjacent to lumen was stained.
Kowalewsky..	Culex Chironomous Ephemerid larvæ Caterpillars	Sodium-indigo-sulphate and	Taken up by cells of tubules and finally excreted into lumen in small crystals. Nucleus not stained.
	Orthoptera	Carminic acid	Not taken up by tubules but by pericardial cells which are arranged in strands and seem to store up foreign matters of various kinds.
Kowalewsky and Cuenot.	Mole cricket	Indigo-carmine Vesuvin	Eliminated by yellow tubules. Eliminated by white tubules.

The presence of uric acid in the bodies of silkworms had been noted as early as 1783 by Chaussier. Others obtained similar results upon examination of other insects, until now it is generally accepted as a fact that uric acid is the main excreted substance of the insects. In a few cases it has been claimed that urates of Na, K, Mg, NH_3, and Ca are found in the place of uric acid. Also there are found phosphoric acid, calcium carbonate, and magnesium sulphate.

Theories of Kidney Action.—Bowman (1842) looked upon the water secretion coming from the glomerulus of the kidney as a means of flushing out the tubules in which urea and other excretory substances were excreted in solid form. He evidently believed, though he did not clearly state, that salts, sugar, and albumin pass into the urine through the capillary walls of the glomerulus. In 1874 and in 1883 Heidenhain presented experimental evidence in support of Bowman's hypothesis, which has since been known as the Bowman-Heidenhain theory. He believed that excretion is accomplished by the vital activity of the epithelial cells of the capsule and of the tubules, and indicated the particular rôles played by the epithelia of the different parts of the structure, as follows:

Glomerular capsule.....	Secretes water and those salts which accompany water everywhere in the organism, such as NaCl, and sugar in glycosuria, and egg albumen.
Convoluted tubules and the wide part of Henle's loop.	Excrete most of the solids found in the urine: urea, uric acid, hippuric acid, pigments, hæmoglobin, and salts. These are accompanied by a minimal amount of water. During diuresis water is excreted in excess by cells of tubules and not by glomerulus.

In other words, Heidenhain looks upon the glomerular capsule as excreting a fluid very similar to blood plasma, except for the proteins which it does not allow to pass. The fluid receives from the cells of the tubules as it passes along their contribution of urea, uric acid, and salts, and finally emerges from the collecting tubes as urine.

Ludwig (1844) took a different view of excretion. He believed that the glomerulus is simply a filter and that all constituents of the blood plasma except the proteins may pass through it freely. Much of the fluid passed off by the glomerulus is reabsorbed by the cells of the tubules, the remnant passing through the collecting tube being the urine.

More recently, through the work of A. R. Cushny and others, there has come to be a modified view of excretion, which is somewhat of the nature of a compromise.

It is now very generally recognized that the glomerulus of the kidney is a filter and that the liquid which passes from the blood vessels into the capsule is a filtrate. The process of filtration is dependent upon:

1. Differences in pressure of the liquid on the two sides of the membrane. Other things being equal, the amount of water excreted through the glomerulus varies with the difference in pressure on opposite sides of the membrane.

2. The character of the membrane. The membrane may vary in permeability from individual to individual, and in the same individual from time to time. These differences in permeability may be due to variations in the arrangements of the molecules in the membrane, and thus of the intermolecular spaces or pores through which the filtration must take place. These differences would affect the amount of liquid filtered during the course of a day and might also modify the character of the filtrate by allowing, under abnormal conditions, the passing of certain substances which under normal conditions would be retained. Modifications of the membrane through lack of oxygen, changes in the hydrogen-ion concentration of the fluid, variations in the Na/Ca balance of the blood, and variations in the concentration of other substances in the blood are factors which may influence the permeability of the membrane.

3. The character of the liquid to be filtered. In addition to the effects of the composition of the blood upon the character of the membrane there are other ways in which the composition may affect the work done by the kidney. The proteins of the blood plasma oppose any separation of the other constituents of the blood plasma from themselves by filtration. The amount of the force involved is variously estimated by different investigators from 20 to 34 millimeters pressure of mercury. This force must be overcome by a superior pressure if filtration is to occur.

Absorption by Kidney Tubule. The filtrate from the glomerulus is an exceedingly dilute solution of such substances as appear in the blood. As the filtrate emerges from the tubule it is found that there is present less water than passed through the glomerular, capillary walls. Also certain dissolved substances are taken up by the cells of the tubules, while others are rejected by the cells and pass off through the ureters. The relative proportions in which certain substances occur in human blood plasma and in human urine are shown in Table 51, taken from Cushny's monograph.

TABLE 51

	Blood plasma, per cent	Urine, per cent	Change in concentration in kidney
Water	90–93	95	
Proteins, fats, and other colloids	7–9		
Dextrose	0.1		
Urea	0.03	2	60
Uric acid	0.002	0.05	25
Na	0.32	0.35	1
K	0.02	0.15	7
NH_4	0.001	0.04	40
Ca	0.008	0.015	2
Mg	0.0025	0.006	2
Cl	0.37	0.6	2
PO_4	0.009	0.27	30
SO_4	0.003	0.18	60

The relative proportions in which the different substances appear in the urine vary much with changing conditions. The water content of urine varies with the amount of water taken in by the individual, and with the amount of water eliminated through the skin and through the intestine. Usually enough water is retained, except among the herbivora, to keep the various solids of the urine in solution. If large amounts of water are taken the depression of the freezing point of the urine tends to be very slight. The depression of the freezing point of blood is about 0.56°C. That of a very dilute urine may be only 0.075°C., while that of a concentrated urine may reach −5°C., the average values ranging from −1° to −2.5°C.

Threshold Bodies.—Cushny has called attention to the fact that certain substances are excreted only when they occur in the blood plasma above a certain concentration, which may be termed the *threshold value.* Other substances are excreted in proportion to the absolute amount of substance present in the plasma. If the substances excreted by the kidney are arranged in a series in the order in which they are concentrated in

the kidney, there will be obtained, first, a large amount of water, then the following:

Urea, Foreign bodies, Sulphates, Phosphates, Ammonia	These occur in greater concentration in urine than in blood. Their elimination is directly dependent upon the absolute amount occurring in the plasma. They are *no-threshold* bodies.
Potassium, Urates	Have a threshold higher than that of chloride, but lower than that of urea.
Chloride, Sodium, Dextrose	Excretion has no direct relation to their absolute concentration in the plasma. Definite threshold value in plasma, below which they do not pass into the urine. *Threshold* bodies.

As the watery filtrate passes down the tubule the gland cells forming the wall of the tubule absorb a portion of the water and return it to the blood stream. The amount of water thus conserved for the body is influenced by a number of factors, and may be from 50 to 99 per cent of the amount filtered from the glomerulus. Some of the solids of the urine are also absorbed from the filtrate. Sodium chloride and sodium bromide, sugar, amino acids, and other food substances are absorbed freely, potassium less rapidly, and sulphates very slowly if at all. These threshold bodies are absorbed in definite proportions, and these proportions are determined by the normal value in the blood plasma.

CHAPTER XXVIII

THE NERVOUS SYSTEM

What is customarily called the nervous system is really a complex of receptors and adjustors. It will be recalled that, among fundamental physiological properties of living matter, irritability and conductivity were mentioned as among the most important. In the higher animals two types of tissues are found which have developed these two properties to a high degree. These are the muscular tissue and the nervous tissue. In muscular tissue the property of contractility is also developed to a very high degree in the highly differentiated forms, and to a lesser extent in the lower animals.

The question may be raised as to why muscle cells may not be stimulated directly by the forces acting upon the organism. In animals in which the muscular elements are superficially placed, such stimulation might easily occur, and apparently does occur among the sponges, and possibly among the Cœlenterates. At any rate, there is not, so far as the author is aware, evidence of anything of the nature of a nerve net to be found among the sponges. Such nets do occur in the Cœlenterates, but here it has not been demonstrated that direct muscular stimulation by the conditions of the environment may not occur. In the higher animals there is also evidence of the direct stimulation of the ciliary muscle of the iris of the eye by light. In the great majority of cases, however, the muscle cells are in a way screened from the effects of the ordinary stimuli which impinge upon the organism. The thick layers of the skin, as well as the bulk of the muscular tissue itself and of other tissues, serve to block off from the deeper-lying cells such impulses as might easily affect cells in the superficial layer. Also the liquid medium by which the muscle cells are bathed is of such a chemical nature as not to stimulate the muscle cells to action. Loeb has shown that slight modifications of the bathing solution may have a decidedly stimulating effect. The suggestion that there may be conduction from one muscle cell to another, without the intervention of any nervous elements, has also been made. Such transmission would, of course, be slow, and for the higher organisms very inefficient, but it undoubtedly occurs in many instances.

Need for a Nervous System.—Living organisms are, in the main, complexes of cells carrying on their vital activities in an environment subject to modification. The simplest existing organisms have but little need for an organ system whose main object is to receive stimuli from the

outside and to meet the changing conditions implied in these by motor activities. Yet it is found, even among the protozoa, that such receptive, conductive, coordinating, and effecting systems have their place. The organism is placed in an environment which may vary in its water or gas content in either quantitative or qualitative ways. It must, if it is to survive, respond to the stimulation of the want of food, or to the presence of certain forms of food; it must respond to variations of temperature, of light, of gravity, of density of water, of pressure, of chemical substances, and to vibrations of air or water, if it is to ward off the attacks of enemies, to secure a food supply, and to continue its race.

Among the lower forms there is no differentiation of organs for the reception of the different classes of stimuli. The protoplasm appears to be all of one kind. All the differing forms of stimuli which impinge upon the body's surface may be imagined to be about equally effective. There are a great variety of environmental conditions to be encountered by the living organism and these must lead to responses on the part of the irritable protoplasm. Among the higher animals a mechanism is provided in the nervous system, with its associated sense organs, for the reception of a wide range of stimuli, both as to quality and intensity, and for rapid conduction from point to point in the organism.

The success of any protoplasmic line will depend upon the degree of discrimination with which its individuals may detect minute changes in their environment, and then their ability to adjust themselves to the conditions of their environment. The behavior of the organism, using the term "behavior" in its broadest sense, may be conceived to be the resultant of *all* the forces coming to bear upon the protoplasm of an individual. These forces may be classified in two main catagories as follows:

External factors:
1. Moisture
2. Light
3. Temperature
4. Gravity
5. Chemical composition of environment:
   - Oxygen tension
   - Carbon dioxide tension
   - H-ion concentration
   - Salt balance
6. Density of medium
7. Contact
8. Pressure
9. Electrical conditions of environment

Internal factors:
1. Heredity
2. Memories—changes in cells as result of experience
3. Habit formations
4. Modifications due to metabolic condition:
   - Variations in metabolic rate

Pathological disturbances of metabolism
Excess of food
Want of food
Want of oxygen
Want of some particular food substance
Production or accumulation of wastes

5. Modifications due to variations in glandular activity
6. Fatigue—exhaustion
7. Parental care
8. Sex urge
9. Turgor of internal parts
10. Pain

It must be recognized that the organism is at all times being played upon by a vast array of forces which act upon it from all directions. It must be recognized, also, that not all of the forces which are operative in the environment make any definite impress upon the organism. While the organism has become adapted to receive a considerable variety of impressions, there are many other manifestations of energy which the cells of the body are not adapted to receive, such, for example, as the waves of the radiophone.

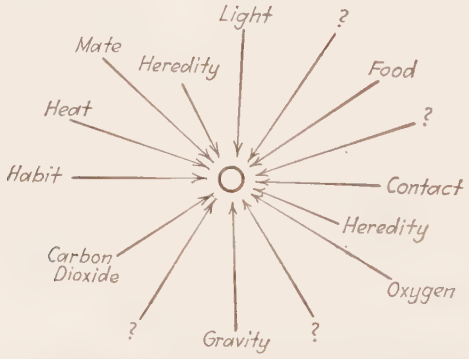


FIG. 91.—Diagram to illustrate the forces acting upon an organism from within and without and which determine behavior.

Of the factors which tend to determine or modify behavior, the external factors have received most attention. This is undoubtedly because they are more or less tangible and subject to control. The internal factors are less tangible, less subject to experimental control, but no less real. Because they seem intangible and difficult to control, many investigators have seemed inclined to leave them out of consideration, or to dismiss them with the suggestion that we can never hope to understand them.

The organism may then be represented as being an object at the center of a sphere of influences at the periphery of which are forces acting upon it from various directions. The direction in which the organism

moves or the activity which it assumes at any moment of time may be considered to be the resultant of all the forces internal and external acting at that moment (see Fig. 91).

Vitalistic View of Animal Behavior.—Students of animal behavior have found themselves arrayed in two opposing groups. These are spoken of as the Mechanists and the Vitalists. The Vitalists believe that the organism is a machine plus certain unknown and unknowable factors. They recognize that the behavior of a purely physicochemical system, operating under fixed conditions, and under purely physical and chemical laws, should be definitely predictable. When the behavior of an organism is observed, however, it is found to be exceedingly difficult to account for all the features of its behavior, so it has been assumed by some that there is in the organism some immaterial directing force or energy which operates upon the organic mechanism in a directing or controlling way, so as to render its behavior more or less independent of physical and chemical laws. The statement is also made that the organism is constantly changing, exhibiting at different times what have been spoken of as differing physiological states, or at least dissimilar responses to given forms and intensities of stimuli.

The difficulties involved in a complete analysis of the factors which comprise the environment of an organism are very great. There are some factors which can readily be discovered, but there are some of so subtle a nature as to be almost beyond recognition and control. Very slight differences in the chemical composition of a medium in which aquatic forms of animals may be living may be sufficient to reverse the form of behavior exhibited by the animal under investigation. These differences are exceedingly difficult to detect, but they must be detected if observational work upon behavior is to be of value. The behavior observed must be that of an animal under a known set of physical and chemical conditions. Unless the conditions are known and can be duplicated, the work is useless; and, if this is true of the environment, how much more it is true in the case of the organism. The nature of the mechanism which reacts under the influence of the more or less stable environment of experiment is not known, nor is anything known of the physical and chemical changes which may take place within the organism as the result of activity. In some cases, some rather shrewd guesses as to the effects upon the organism of certain environmental conditions have been made, but any complete physicochemical account of behavior is far from having been elucidated.

It has been very easy for biologists to cover up their ignorance as to things which may be really explainable by an appeal to an entelechy or the elan vitale. Until comparatively recent years, biologists have not been willing to recognize that physical and chemical operations lie at the foundation of the explanations of animal activities, nor to confess their

own ignorance of these subjects. If the Vitalist really feels the necessity of maintaining the integrity of the entelechy or other controlling vital influence, he should make use of the line of attack of the Mechanist and demonstrate by the means of physics and chemistry the inadequacy of the mechanistic point of view.

Mechanistic View of Animal Behavior.—The Mechanist looks upon the organism as a machine, surrounded by a material environment, under the influence of which it reacts. The machine may be complex, and undoubtedly is. The materials of which it is composed are the materials of physics and chemistry. The laws which control the machine in its operations and activities are the laws of physics and chemistry. The whole behavior of the organism is held to be the resultant of the physical and chemical forces acting upon the material mechanism which is called the organism. It is also recognized by the Mechanist that the machine is subject to modification as the result of the activities which it assumes. After such modification, the machine is, then, a somewhat different machine from what it was before the modification, and as it is different it must respond to stimulation in a different manner. These modifications are, however, to be considered simply as the results of physical and chemical processes going on within the organism.

It happens that students of animal behavior have not found it easy to account for the behavior of any form of animal under all the conditions in which it may find itself. There may be a variety of reasons for this difficulty. One reason certainly is the lack of information available as to the nature of the physicochemical machine called the organism, and as complete knowledge as to its nature is not had, there is even more doubt as to the constantly shifting structure and composition which it must assume under differing conditions, that is, under stimulation. There is also a very fragmentary knowledge of the physical and chemical laws and forces at work in and upon the organism. There is probably no group of scientific workers any more aware of the limitations of knowledge in their own field than the physicists and chemists, nor who appreciate more keenly the difficulties involved in pushing back the bounds of knowledge. Since there are very definite limits to physical and chemical information, it is not to be wondered at that there are also limits in the application of the knowledge of physics and chemistry to physiological matters. Every advance in physics and chemistry may be significant for a more complete understanding of the living organism, so it is of importance that the biologists seek to encourage the physicist and the chemist in their endeavors to push back the horizon of knowledge.

There are a good many biologists who are unable to accept either the purely mechanistic or purely vitalistic conception of vital phenomena. There are some, like Dr. Benjamin Moore, who find it necessary to postulate a different form of energy, biotic energy, to explain vital processes.

There are some who at times support the mechanistic conception—and then a little later find themselves in favor of some of the tenets of the Vitalists. It is probably too soon to say that in either belief all of the truth is to be found.

Some biologists, among whom Parker is a conspicuous example, hold that complexity of type of organization is a factor which must be taken into account in the determination of behavior. In a simple reflex mechanism the responses must, of necessity, follow a perfectly definite order, and be definitely predictable. A complex mechanism, on the other hand, may possess a variety of possible pathways over which impulses may travel. If the impulses travel over one pathway, a definite response follows; if over another pathway, another—but no less definite—response occurs. The nature of the response depends, then, upon which particular pathways are involved in carrying the impulses. It is entirely within reason, also, that the individual may have the power to determine over which pathways impulses must travel. This means freedom of choice.

There can be no doubt that following a given stimulus many types of response are possible. The determination of the pathway may be at first a matter of chance, or it may be due to training. In either case the pathway once established by repetition comes to be the natural channel for the passage of impulses. Thus, the response may come to be of the nature of a reflex. It is possible for the organism to shift the impulse along some other pathway. This constitutes choice or freedom. Parker's view is materialistic in its nature, though not one which would be readily accepted by many members of the mechanistic school.

To understand the way in which various simple movements are brought about is not easy. To explain complicated movements of behavior calls for a large amount of highly specialized information. It is not the purpose in this course to do more than indicate the fundamental structural relations which have been found to exist in a variety of animal groups, and to show how these may operate in the animals concerned, in the hope that the principles thus established may give a clue to the operation of such a highly complex system as is found in the human being.

Neuromuscular Apparatus of the Protozoa.—It has been customary to think of the protozoa as forms which exhibit little of the differentiation which is to be found in the more complex multicellular animals. In particular the belief that the protoplasm of the protozoa is almost uniformly conductive and contractile seemed well founded. That this is not the case has been shown in a series of brilliant investigations carried on at the University of California under the direction of Professor Kofoid. Attempts had previously been made by others to differentiate nervous elements in protozoan cells, but with only partial success. With the work of Sharp (1914) upon *Diplodinium ecaudatum*, a very complex

member of the Oligotricha, it became evident that an exceedingly complex neuromuscular structure may exist within the limits of a single cell. Yocum (1918) described a similar system in *Euplotes*, and Taylor (1920) demonstrated that the system in *Euplotes* is conductile rather than supporting or contractile, and that the conductive properties function in the coordination of the movements of the locomotor organelles with which the system is intimately associated.

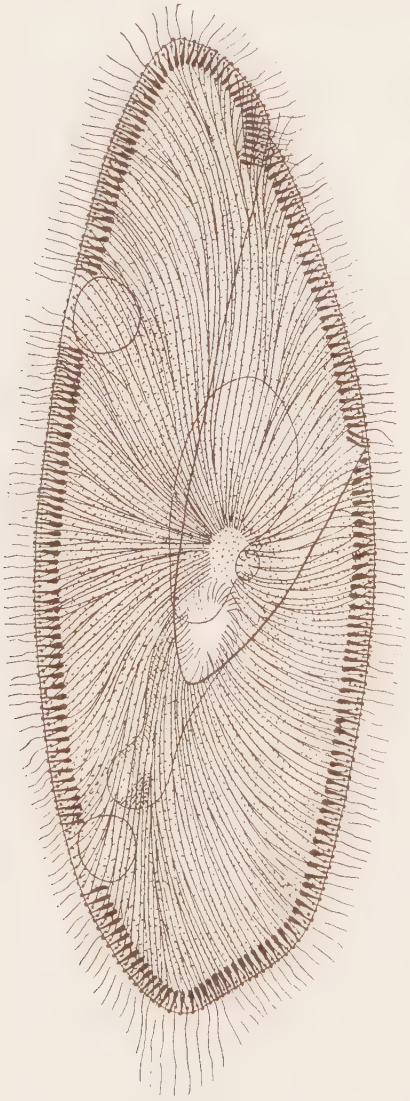


Fig. 92.—Neuro-muscular system of *Paramœcium*. (Note that fibers connect the nerve centers with every cilium and trichocyst.) (*According to Rees.*)

Rees also (1922) reinvestigated Paramœcium and has found a most elaborately organized neuromotor system. The system in Paramœcium (Fig. 92) consists of a neuromotor center lying at the anterior end of the cytostome, and two peripheral whorls of fibers, the larger on the oral side, the smaller on the aboral side, and of two other groups, the cyto pharyngeal fibers, one running to the anterior zone of membranelles in the cytopharynx, and the other around the margin of the cytostome to the posterior membranelle zone. All the fibers of the peripheral whorls and of the cytopharynx ramify out from the neuromotor center at the anterior end of the cytostome. The radiating fibers connect at their outer ends with the basal granules of the cilia and with the trichocysts.

Nervous Organization.—In all groups of animals above the sponges, there are definite mechanisms given over to the transmission of impulses. These vary greatly in the complexity of their design and in the degree to which specialization has taken place in the component parts. The simpler of these mechanisms may be called *nerve nets* and the more complex, *synaptic nervous systems.*

The nerve net is a system made up of very primitive units which have been termed *protoneurones* by Parker, that is, cells with processes

which have protoplasmic continuity with neighboring similar cells and with effector cells. This type of system is characterized by diffuse transmission of impulses, that is, the cells, being spread out in the form of a network, tend to pass on from cell to cell throughout the whole system any impulse which may affect any cell.

The *synaptic nervous system* is made up of *neurones,* cells with processes not coming into direct contact with similar cells, but acting through what are known as synapses. Here, the conduction is ordinarily in only one direction in any cell fiber, and hence is spoken of as *polarized.*

The Nerve Net.—The nerve net is a type of structure characteristic of the Cœlenterates generally, though there are, of course, variations in

FIG. 93.—Nerve net of *Hydra fusca.* (*Drawing made by Helen Daniels Young from a preparation by Powers and Powers.*)

the degree to which independence of the different cells composing the network has been developed. The nerve net is also to be found among the Echinoderma, worms, Crustacea, Insecta, Mollusca, and Vertebrata, being in these higher forms present in the walls of the blood vessels and of the alimentary tract. Parker calls attention to the fact that the gradually diminishing appearance of this type of nervous organization, as the animal scale is passed in review, seems to indicate that it is the more primitive type of structure, and that it has gradually given way to the synaptic system in the higher animals.

The structure of the nerve net may be illustrated by a reference to the conditions found in Hydra and similar forms. Ganglion cells and nerve fibers occur everywhere under the epithelium, and sometimes even in the epithelium. These cells and the fibers arising from them have been termed *protoneurones*. They form a delicate network as shown in Fig.

FIG. 94.—Ectodermal nerve plexus of *Hydra fusca*. The parallel lines indicate the muscle fibers upon the supporting layer. (*According to Schneider.*)

93. Here, it will be noted that there are definite protoplasmic connections between near-lying cells. The particular preparation from which this drawing was made does not show the fibers running to the effector cells, nor the fibers running to the ganglion cells from the receptor cells.

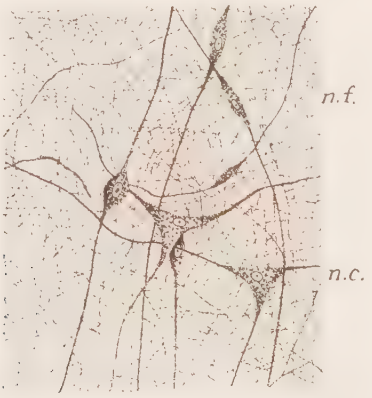


FIG. 95.—Nerve plexus about the mouth of *Anthea cereus*. *n.c.*, nerve cell; *c.f.*, nerve fiber. (*According to Hertwig, from Schneider.*)

Such fibers must, of course, exist, and have been illustrated by Schneider (Fig. 94). The cells of this network are rather evenly distributed throughout the body wall of Hydra and so serve to throw all parts of the body into correlation. It is apparent that impulses originating in any part of the body may be transmitted in all directions through the

network, and so come to involve all parts of the body. Inasmuch as the pathway, normally, from the receptor to the contractile element is exceedingly short, the passage of the impulse from nerve-end organ to the effector may be said to be direct. At any rate there is a distinct protoplasmic continuity.

Hertwig has shown that in the Actinian *Anthea cereus* there is a definite plexus of nerve elements about the oral region. Whether these elements form a definite nerve net is a matter of some doubt. It seems more likely that the condition existing in this form is that of a plexus of nerve cells with long fibers which branch and anastomose in various ways and, perhaps, come into contact relations with each other (see Fig. 95).

A *B*

FIG. 96.—*A*. Two nerve cells of the sub-epithelial nerve-net of the palate of the frog. *B*. Nerve-net of a small artery of the frog.

Evidence is not lacking that, even high up among the vertebrates, structures exist which are comparable to the nerve nets found among the Cœlenterata. The nerve nets found in the walls of blood vessels are familiar examples. Possibly, also, the nerve plexuses of the alimentary walls of many animals belong in this same category. Examples of two such nerve-net structures are shown in Fig. 96.

The nerve net posesses certain distinguishing features which deserve note:

1. The cells of the nerve net are not only in physiological but in structural continuity.

2. The cells of the nerve net are in intimate relations with the muscle cells which they excite.

3. In transmission, impulses flow more freely into regions where the net is stretched than into regions where it is not stretched.

4. The nerve net provides for organic autonomy.

5. Transmission in the nerve net is diffuse.

6. The power of the nerve net to bring about coordinated movements depends upon its refractory phase, *i.e.*, impulses brought to bear upon the cells during their period of activity are not effective in inducing reactions.

7. Evidences of polarity in the nerve net indicate that the net tends to become converted into a synaptic system.

8. Modifications of the nerve net of such a sort as to render the fibrils elongated so as to place the nerve cells at some distance from the muscular cells, and the fibrils to become aggegated in trunks or bands, mark a change from a true nerve net to a synaptic system.

Transition from Nerve Net to Synaptic System.—Parker has called attention to the evolutionary change from the nerve net to the synaptic system. The first factor in this evolution is the tendency assumed by the cells to become polarized, that is, to conduct impulses in one direction only. The earliest indication of this polarity may be that in which conduction occurs more readily in stretched than in unstretched regions. A second factor in the evolution is the lengthening in one direction of the fibers leading from the sensory ending to the motor cell, and the distribution of such fibers to specific effector cells, rather than indiscriminately through a widespread net. Parker suggests that the lengthening of the fibers, so that the cells which were originally close together have come to be widely separated from each other, accounts for the origin of the synapse. As the cells migrated from each other they lost contact with each other. In the attempt to reestablish connections, they have been but partially successful.

A. R. Moore (following the method employed by Langley) has presented evidence of a physiological character indicating a rather regular progression in the development of the synapse in different animal phyla by the use of strychnine. This drug has the property of increasing the reflex excitability of many animals, and it has been assumed that this effect is the result of a lowering of synaptic resistance. The drug has no effect upon the neuromuscular responses of the Cœlenterates, slight effects upon the responses of Echinoderms, and much greater effects upon the responses of Mollusca and Crustacea.

Chemical Differentiation in the Nervous Systems of Invertebrates.—Moore has called attention to the fact that the differentiations of the nervous systems of the invertebrates are not alone structural in character. Here and there in the animal kingdom there is evidence that the nervous tissues show evidence of chemical differentiation. It would, of course, be natural to expect to find something of this sort if there has been any

real evolution in the animal kingdom. Moore found that in the cephalopod *Loligo pealii* caffeine brings about a hypersensitivity of the cerebral ganglia, while camphor affects the stellar ganglia alone in the same sense. Atropine causes spasms in the squid, but inhibits the activity of the chromatophores. Camphor shows a selective action on the central nervous system of the shrimp, *Crangon vulgaris*, paralyzing the elements which function in backward swimming movements and exciting those which control forward movements.

The Neuron Theory.—The present understanding is that the nervous systems of all the higher animals are composed of units called *neurones*. This idea had its origin in the work of Golgi (1882–1885) and has been supplemented and confirmed by the studies of many investigators. The neuron is a nerve cell (Fig. 97) together with all the protoplasmic processes which arise from it. It is probably the most highly differentiated type of cell. It has to perform all of the fundamental physiological functions which are a part of the activity of any other kind of cell, but it probably has rather more than the common burden in this regard, because of the large mass of the cell body and the great length of its many processes. In addition to the general functions involved in the maintenance of its protoplasmic structure, it has to perform highly developed special functions. The nerve cell is more highly differentiated not only in form, but in its internal structure and chemical and physical balance. Its protoplasm is in a condition of extreme irritability and instability.

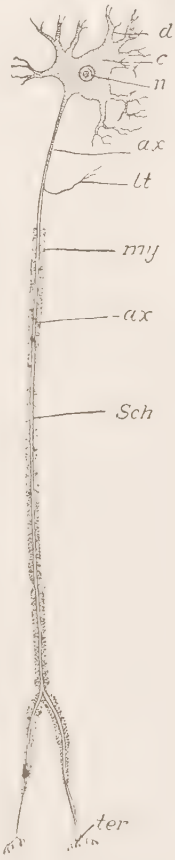


FIG. 97.—Diagrammatic representation of a vertebrate neuron. *d*, dendrites; *c*, cell body; *n*, nucleus; *ax*, axon; *lt*, lateral process from axon; *my*, myelin sheath; *Sch*, Schwann's sheath; *ter*, nerve ending. (*From Schneider.*)

There is a great deal of variation in the structure and physicochemical balance of neurones associated with position in the animal kingdom, and the particular rôles which they may play in the life of the individual. While there is evidence of a great advance in structural complexity of the individual cells in the progress from lower to higher forms, there is undoubtedly a more striking increase in the complexity of arrangement of the neurones in the building up of the more complicated nervous systems. There is also, less evident to be sure, a very great increase in the degree of irritability and instability as one passes from lower to higher forms.

In a brief statement of the essentials of the neuron theory, in general, the statement of Heidenhain may be followed.

1. The neuron of the adult body is an anatomical unit, and corresponds to a single cell.

2. The neuron is a genetic unit and had its origin in a single embryonic cell.

3. The nervous system, as a whole, is made up of these units, very systematically arranged and organized, and these elements are the only elements in the nervous system which play any part in nervous activities.

4. The neurones are not only morphologically distinct and separate in origin, but they remain so during life. They do not at any time have any protoplasmic continuity or fusion with each other.

5. The neuron is a trophic unit. Any event which affects the metabolic processes of any part of the unit affects the whole, but does not directly affect units adjacent to it. Destruction of the nucleus and cell body destroys the whole neuron.

6. The neuron is the functional unit of the nervous system.

There is a great deal of structural variation among neurons according to their position in the animal kingdom and the particular part which each is to play in the life of the individual. They have been variously classified as to form, as monopolar, bipolar, multipolar, etc. cells. From a physiological point of view, this seems less important than to recognize the rôles which the neurones play in receiving stimuli from without and transmitting impulses toward the nervous centers (*afferent neurones*), carrying impulses from a cell body located in the central system to some effector structure such as muscle fiber or gland cell (*efferent neurones*), or in transferring impulses from one neuron to another within the same central system (*internuncial, or adjustor, neurones*).

The relative merits of the nervous systems of the higher and lower animals lie not alone in the larger number of neurones involved in the structure of the nervous system of the higher animal, but also in the fact that each individual neuron is a more complex structure, having a larger number of highly branched processes; while in the lower animal there is a smaller number of processes with fewer branches, and, so, less possibility of entering into close relations with a large number of cells.

Each neuron has projecting from the cell body two kinds of processes. The first of these are the *dendrites* (Fig. 98). These are very much branched and form a shrub-like mass and conduct impulses toward the cell body. The other type of process is the *neurite*, usually a single long process which conducts impulses outward from the cell. The neurite is usually very little branched, such branches as do arise coming off at right angles to the main axis, until at its distal end it breaks up into a terminal brush of minute fibrils.

The Synapse.—Between the protoneurones which constitute the nerve net of the lower metazoa, there appears to be definite protoplasmic

continuity, the protoplasm of one cell being definitely continuous with the protoplasm of its neighboring cells. This is not now thought to be true in so far as the neurons of the nervous systems of the higher animals are concerned. Here, the processes of the cells do not have continuity (Fig. 99), though there may be contact. There is, however, a form of physiological continuity, so that an impulse may be transmitted from one cell to another, analogous, perhaps, to the way in which induction currents are set up in an induction coil. The centrifugal neuritic branches come into contact or close relations with the much branched centripetal

FIG. 98.

FIG. 99.

FIG. 98.—Pyramidal neuron (Type I of Golgi) from the cerebral cortex of a rabbit. The axon gives off numerous collateral branches close to the cell body and then enters the white substance, within which it extends for a long distance. Only a small part of the axon is included in the drawing. *a*, axon; *b*, white substance; *c*, collateral branches of axon; *d*, chief dendrite; *p*, its terminal branches at the outer surface of the brain. (*From Herrick, after Ramón y Cajal.*)

FIG. 99.—Synapse between an ascending fiber entering the cortex of the cerebellum and the dendrites of a Purkinje cell. (*Redrawn from Herrick, after Ramón y Cajal.*)

dendrites of one or more neurones. No true protoplasmic continuity exists between the cells; there is always between the protoplasms of the two cells the barrier of two cell membranes. These, together with any material which may lie between them, constitute the *synaptic membrane*. There is consequently no passage from one cell to another of neurofibrillæ.

Integration of Animal Bodies.—It has been seen how, in the protozoan, the different portions of the body come to be integrated into a unified, purposive, machine through the medium of the so-called neuromotor apparatus.

In the multicellular animal there comes, along with increasing differentiation of parts, a corresponding increase in the need for some means by which the different parts and activities of the organism shall be coordinated. Team work on the part of the different organs and tissues of the animal body is essential if the organism is to be successful in its living; and this team work must extend not only to the larger and more conspicuous organs, but even to the individual cells which make up the organ.

That complexity of organization may be attained in a variety of ways will be recognized; there may be considerable differentiation of parts, even within the limits of a single cell, as seen in the neuromotor apparatus of the higher protozoa. There may be extreme differentiation between the cells composing the bodies of the multicellular animals; or instances may be found where there is an extreme of cellular differentiation coupled with an extreme of intracellular differentiation, as is certainly true in the nerve cells of the higher animals. There can be no question that the individual cells of the sponge are less highly developed than the single cells of some of the protozoa, and yet there is a tendency to look upon the sponge as a higher form than the protozoan because of its multicellular condition, and the occurrence in it of cells of different sorts, each with its own special work to do.

Two principal methods of establishing correlations between different and distant portions of the animal body have been established:

1. There has been developed a more or less highly differentiated nervous system, whose control extends not only to the activities of organs, but may involve individual cells.

2. There has been developed a chemical control of body tissues through the production, in the tissues of the body, of substances of the drug type.

Of these two methods the earlier to be developed was undoubtedly the nervous system.

It is recognized that the nervous system is not a mechanism which is entirely independent of other structures in the body, but that it is at all times in more or less direct association with the various specialized cells which act as the receptors of impulses from without, on the one hand, and with the various effector cells, such as gland cells and muscle cells, on the other. Thus, the central nervous system, commonly so called, is simply one part, a most important part to be sure, of a receptor-adjustor-effector mechanism, a neuromotor apparatus.

It will be discovered that there are great variations in the degree to which certain types of nervous elements have become incorporated within the bounds of the central nervous system.

Range of Ethereal Vibrations.—If the various wave lengths of all the various ethereal waves, from the shortest Millikan waves and radium waves, up to the longest Hertzian waves, should be plotted in the form of a spectrum, it would be noticed that the part of the spectrum which is

visible occupies only a very small part of the whole. There is apparently no other portion of this spectral range for which the human body has developed any receptors at all. There is a possibility that the skin may contain receptors sensitive to some of the infra-red rays; but, in general, it may be said that our temperature sensations come from some change in the rate at which heat is being radiated from the body, or from the impact of radiations of various sorts upon the body surface, with the result that there is a blocking of the radiation and a degradation of the energy into heat. For all the rays, from the Millikan up to the violet rays, and from the extreme border of the red out to the long Hertzian waves, we have no organs of perception. The spectrum (Fig. 100) is an attempt to convey some idea of the very narrow limits of the visible spectrum as compared with the total range of ethereal waves. It is to be noted that, in passing from the Millikan waves toward the other end of the scale, the length of the wave at any scale divison is roughly double that of the next preceding division. Some of the Hertzian waves at the extreme end of the series are as much as 12 kilometers in length.

Classes of Receptors and Their Limitations. Attention has already been directed to the vast number of wave lengths of the vibrations which take place in the ether, and the extremely limited range of such vibrations which animals generally are able to receive. The ethereal vibrations constitute but a small fraction of the total number of impulses which beat upon animal bodies. The cells whose function it is to receive these vibrations are called receptors. The bulk of information in regard to these structures has come from a study of the receptors of human beings. It is not possible to know very much about the actual sensations of the lower animals. From what may be observed of the behavior of the lower animals, the impression is gained that

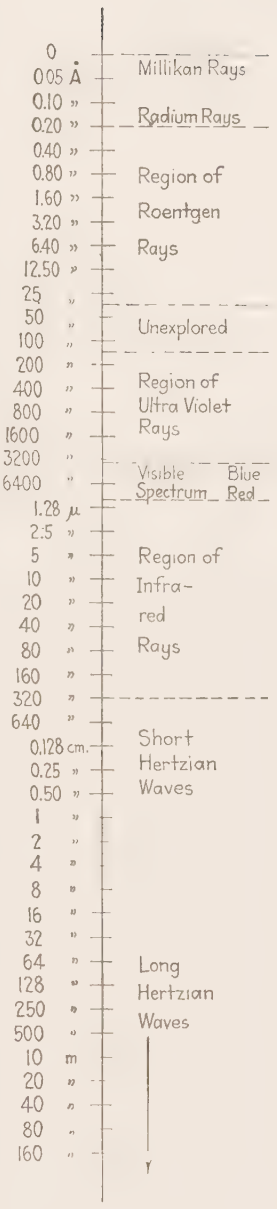


FIG. 100.—A chart[1] of the spectrum of etheral waves ranging from the shortest Millikan rays to the very long Hertzian rays.

[1]Note that the scale values indicating wave lengths double with each division passing from the shortest toward the longest waves. The visible spectrum occupies an almost insignificant portion of the total spectral range.

many of them are sensitive to a great many of the stimuli which affect the human being. It is undoubtedly true that the human being is sensitive to some impulses which make no impression upon many of the other animals. It is equally true that many of the lower animals may receive and respond to stimuli to which human beings are wholly indifferent.

As they occur among higher animals, receptors may be classified as follows:

1. Exteroceptors (somatic receptors):
   a. Chemoreceptors:
      General chemical sense.
      Smell.
      Taste.

It is the function of these receptors to determine the presence in the immediate environment of substances in either the molecular or ionic state.

   b. Contact receptors:
      Touch.
      Pressure.
      Pain.

These are sensitive to contact with the body of solid or liquid substances and may respond to vibrations of the substances from very slow up to 1500 or more per second. The strength of the stimulus may determine whether the sensation be one of touch or pain.

   c. Temperature receptors:
      Heat.
      Cold.

These determine whether some substance in the bodily environment is warmer or colder than the skin. Herrick states that they are able to receive waves having wave lengths of from 7.6×10^{-5} millimeters, to 320 microns, and rates of vibration of from 3×12^{10} to 8×10^{14}. These waves are in the infra-red region of the spectrum.

   d. Sound receptors.

These receive vibrations of air or water of from 12,280 to 13 millimeters in wave length, and of from 30 to 30,000 vibrations per second, and give sensations of sound, or musical tone.

   e. Light receptors.

These are receptors giving sensations of light and color. They have rates of vibration of from 4×10^{14} to 8×10^{14} per second, and wave lengths of from 4×10^{-5} to 7.6×10^{-5} millimeters.

   f. Position receptors.

Receptors usually actuated by the force of gravity, and which give information as to the position of the body with respect to the earth.

2. Interoceptors:
   a. Hunger.

Due to muscular contractions of stomach walls.

b. Thirst.

Sensation felt in throat. Prolonged want of water may give rise to general body sensations which can be relieved only by intake of water.

c. Nausea.

Associated with antiperistalsis.

d. Respiratory sensations:

Suffocation, etc.

e. Circulatory sensations:

Pressure, flushing, heart symptoms, etc.

f. Sexual sensations.

g. Visceral pain.

h. Proprioceptors.

Located in muscles, joints, and tendons.

The bounds of this course do not permit entering into a discussion of the structure and functions of the many different forms of receptors found in different animal groups.

Each of the types of receptors named above responds to a particular type of stimulation, of an intensity far below that which would be required to stimulate the nerve fiber directly, and gives to the organism a degree of information concerning environmental conditions that could not be otherwise obtained. The receptors thus form a mechanism designed to lower the so-called *threshold of stimulation.*

Threshold of Stimulation.—Protoplasmic cells are not only limited in the range of vibrations or wave lengths to which they are sensitive, but they respond only to stimuli of a certain minimum intensity. The minimum intensity required to excite a nerve to activity is spoken of as the *threshold value.* The term may be applied in the case of the stimulation of a receptor by the natural stimulus to which it is attuned, or, in the case of nerve, to the minimum electrical stimulus which will induce a reaction.

The Law of Specific Nerve Energies.—From what has been said it will be surmised that nerve impulses are all fundamentally alike, and yet the sensations which arise as the result of these nerve impulses are not all alike. The specific effects of the excitation of different nerves are due to the differences in the end organs. Stimulation of the optic nerve, for example, always gives the sensation of light, no matter what method of stimulation is employed; similarly, stimulation of the auditory nerve, by whatever method, always gives the sensation of sound; stimulation of the chorda tympani nerve, by whatever method, always gives the sensation of taste. To this type of behavior has been given the rather misleading name, *the law of specific nerve energies,* first enunciated by Müller.

Elementary Functions of the Excitable Tissue.—The nervous tissues may be excited by a variety of stimuli, as has been already pointed out.

The passage of an electrical current through a portion of a nerve or muscle fiber results in a local change which is limited to the region through which the current flows. In order to produce any effects in a remote part, it must attain a certain intensity. If the intensity of the current is sufficient a disturbance will be set up in the fiber which will be conducted from the point of disturbance over the whole length of the fiber. The local change in the fiber has been called by Lucas *the local excitatory process.* The resulting disturbance which passes over the tissue has been named the *propagated disturbance.* This is the phenomenon commonly called the *nerve impulse.*

Although there has been a great deal of investigation of nerve action, as yet there is not a thorough understanding of the nature of this local excitatory process, though there is some knowledge of certain phenomena which attend this excitation. Among the attendant phenomena are electrical changes in the nerve fibers, and the production of small amounts of carbon dioxide. A. V. Hill found that the heat production involved in a single propagated disturbance must be less than one one-hundred-millionth of a degree Celsius. He believes, therefore, that the propagated disturbance is not the result of an oxidative reaction. On the other hand, it has been very generally recognized that oxygen is essential for the continued activity of nervous tissue. It may be worth considering whether there is here an oxidative reaction in which there is a very high efficiency in the production of nervous energy, similar to the higher efficiency of the oxidative process taking place in light-producing organs.

Carbon Dioxide Production by Nerve.—The nature of the nerve impulse and its transmission have been looked upon by many as insoluble mysteries. In fact, up to 1850 it had not been deemed possible even to measure the rate at which the nerve impulse travels; but in that year Helmholtz determined that in the frog the impulse travels at about 29 meters per second. The possibility that the passing of a nerve impulse could be explained in terms of chemical action incited the imaginations of many physiologists. Attempts have been made to show that:

1. Oxygen is necessary for nerve action.
2. Carbon dioxide is produced during nerve action.
3. Changes in the structure of nerve cells occur as the result of nerve action.
4. Heat is given off during nerve action.
5. Electrical changes take place during nerve action. The last one of this list was the first one to be proved. The other statements have received a great deal of attention. There is little doubt that much oxygen is needed by the nervous system, though there is not available quantitative data as to the exact amounts required nor any indication as to how the oxygen is used.

Measurement of the amounts of carbon dioxide produced by the nervous elements during work or rest have proved to be very difficult. Tashiro (1913 and 1917) devised a special apparatus (biometer) by which he believed it possible to detect and measure even the small amount of carbon dioxide given off by a quiescent, or by an active, nerve. The apparatus consisted of a small chamber in which the object to be tested could be placed above a drop of barium hydroxide in an atmosphere free of carbon dioxide. Tashiro contended that, if carbon dioxide were given off, crystals of barium carbonate would be formed on the surface of the drop, and that the production of the crystals would be so regular that the method could be used for the quantitative determination of the carbon dioxide produced. He found that 10 milligrams of nerve of the spider crab gave 6.7×10^{-7} grams of carbon dioxide in 10 minutes; and that, upon stimulation, this amount was increased to 16×10^{-7}, nearly three times as much.

Bayliss was very critical of Tashiro's method and results, and expressed the idea that Tashiro had not been successful in measuring the carbon dioxide of nerve alone. Moore had also attempted a determination of the carbon dioxide production of nerve by means of an indicator method, but was not able to convince himself that there was any increase in the production of carbon dioxide when the nerve was stimulated.

Parker has made a reinvestigation of the whole matter, using several different sorts of nerve. He made use of an indicator method in which he noted the change in color of a solution of phenolsulphonepthtalein from pH 7.78 to pH 7.36. The time required for this shift in color was the time required for the production of a given amount of carbon dioxide, and usually required from 10 to 20 minutes. In the apparatus used the amount of carbon dioxide needed to produce the color change was found to be 0.020064 milligrams. In another type of apparatus used when the nerve was to be stimulated, the amount of carbon dioxide needed to bring about the color change was found to be 0.019866 milligram. The apparatus was found to be sensitive to 0.001 milligram carbon dioxide, and was about one-tenth as sensitive as Tashiro claimed his apparatus to be.

Parker first made use of the lateral line nerve of the dogfish, *Mustelus canis*. This is one of the few pure nerves to be found among the vertebrates. He found that, when the nerve is first placed in the respiratory chamber, there is a rapid discharge of carbon dioxide, and this is followed by a steady outflow which may continue for a number of hours. Parker is of the opinion that the gush of carbon dioxide, when the nerve is first placed in the chamber, is due to carbon dioxide which has saturated the nerve and its liquid environment, and is perhaps being held mechanically by the tissue, and that the steady outflow represents the regular, slow production of carbon dioxide by the nerve. Cutting or crushing the nerve causes a gush of carbon dioxide similar to that which occurs when the

nerve is first placed in the chamber. Ordinary handling of the nerve does not have this effect. Nerve which has been boiled gives off a small amount of carbon dioxide for an hour or two, and then stops. The amount of carbon dioxide produced by a quiescent living lateral line nerve of the dogfish was found to be, on the average, 0.0095 milligram per minute per gram of nerve. This figure is somewhat higher than that obtained by Tashiro, 0.0067 milligram per gram of nerve per minute, in the case of the spider crab, and 0.0055 milligram per gram of nerve per minute in the case of the frog.

Parker found that all nerves showed an increase in carbon dioxide production when stimulated by the electric current, the increase being from 14.3 to 16.7 per cent of the rate in quiescence.

In a similar way Parker investigated the carbon dioxide production in the nerve cord of the lobster, *Homarus americanus*, and found that, when it is fresh, it gives off carbon dioxide at the rate of 0.20 milligram per gram of cord per minute. This amount falls steadily to a value of 0.07 milligram per gram per minute within a few hours, and ceases entirely to give off carbon dioxide with the death of the cord. Stimulation increases the rate of production of the carbon dioxide by about 26 per cent.

In the frog, Parker found that the nervous elements of the sciatic nerve give off carbon dioxide at the rate of 0.008 milligram per minute per gram of nerve. Stimulation increases this amount by about 16 per cent. Parker also estimated the carbon dioxide production of 1 centimeter of an individual nerve fiber and found it to be 0.000,000,042 milligram carbon dioxide (4.2×10^{-8} milligrams). This minute amount he estimates to consist of 578 thousand million molecules of gas.

Fatigue and Exhaustion.—Much emphasis has been given to the claim that it is not possible to fatigue nerve fibers, or even nerve cells, except under most extreme conditions. Several years ago Smallwood and Rogers studied the nerve cells of a number of invertebrates under conditions of high nutrition, starvation, rest, fatigue, etc. They found that in the forms studied there are, in the cytoplasm of the ganglion cells of well-fed and rested animals, numerous, minute, solid granules, often of a yellow or orange color. During starvation or under excessive stimulation these granules become reduced in size and number and their places are taken by small droplets of clear liquid. Because of the very clear appearance of the liquid the spaces in the cell occupied by the droplets were spoken of as vacuoles. It is, in fact, possible to watch the gradual change of phase from granule to liquid droplet in the living cell. This can be accomplished by mounting a living nerve cell between electrodes upon a slide, and subjecting it to electrical stimulation. It is also possible to see, in cells which are allowed to rest after a period of stimulation, the rebuilding of solid granules in some of the vacuoles. These

authors concluded that the granules are substances which are stored in the cells during periods of rest and good nourishment, to serve as sources of energy for the work which the cells may at any time be called upon to do during any period of stress. That they are the immediate source of the energy of nervous action seems doubtful; but that they serve as depots of reserve material seems plausible. According to these authors, nervous fatigue and exhaustion would seem not only to be possible, but to be a natural consequence of unlimited activity in which the catabolism of certain important energy-producing substances occurs more rapidly than their anabolism. The presence of a good food supply is not the only factor involved in the maintenance of the structure of the nerve cells. It has also been found that long-continued stimulation of nerves, under conditions of reduced oxygen supply, leads to failure more quickly than if an ample supply of oxygen is present.

The Nature of Nerve Action.—There is some evidence in favor of the idea that the activity of the nerve cell in sending out a nerve impulse is analogous to the activity of the muscle fiber. There is a period of activity, followed by a period of recovery. In the muscle, these periods are of sufficient length so that they may readily be measured. In the nerve, they must be exceedingly short. It is well known that the wing muscles of insects contract at the rate of 300 to 400 times per second. This rate is maintained apparently without fatigue. Though the interval between one contraction and the next is very short, it is long enough to provide for those periods of activity and recovery which occur in the nerve cells which call the muscle fibers into action. Allowing 300 contractions per second would mean that there must be in the nerve cell the potentiality to repeat the whole cycle at least 300 times per second. As a matter of fact, it is doubtful if the contractions of the wing muscles of the insects are to be considered as series of single muscle twitches. They are more likely to be of the tetanic type, though of very short duration. If this is true, each contraction is likely to be the result of the application to the muscle of several stimuli, rather than only one.

G. F. Göthlin has suggested that the nerve impulse is a phenomenon which involves both physical and chemical actions. He thinks that the Nissl granules of the nerve cells are arranged in a definite series, each granule acting as the starting point of a local chemical reaction which gives origin to a wave of change propagated along the nerve fiber. By a succession of these waves of change, often as many as 136 per second, the normal nerve impulses are produced. He regards the nerve fiber as a passive conductor, the fibers of the axis cylinder marking the paths followed by the chemical changes. He thinks of the medullated sheath simply as an insulator, and holds that the fibers with the thicker sheaths are those of the more rapid transmission.

Rate of Discharge of Motor Nerve Cells.—There is evidence tending to show that stimuli from the motor nerve cells enter the muscles at a very rapid rate. It is, of course, recognized that the ordinary contractions of skeletal muscles are of the sustained or tetanic type. In order to bring about such contractions, stimuli must enter the muscles at very short intervals. Various methods have been devised to give information as to the frequency of the impulses coming into the muscles. From a study of the electrical-action currents of muscle, Piper came to the conclusion that motor impulses must enter human muscle at the rate of about 50 per second. Forbes has made use of a telephonic method which has given important results. He believes that Piper's figure is too low and that the true value is not less than 300 per second, and that it may be as much as 1000 in certain cases. In the frog muscle the rate seems to be about 200 per second, and in the muscle of Mytilus about 300 per second. Garrey has found in the heart of Limulus a much slower rate of discharge —12 per second. Whether this figure actually represents the rate at which the nerve impulses enter the muscle seems a matter of some doubt. As the discussion proceeds, it is found that each of the vibrations of the muscle involved in its contraction is associated with a separate and distinct nervous impulse sent into the muscle; and not only that, but each nerve impulse is a complex of several phases—local excitatory process, refractory period or phase, period of recovery, etc.

The Nerve Impulse.—The period of activity of the nerve fiber includes the *local excitatory process*, which must have a certain minimum degree of intensity, and the *propagated disturbance*. Following these, there is an *absolute refractory period* during which the nerve is immune to all forms of stimulation. As the nerve begins to emerge from the absolute refractory state, it is still partially immune to stimulation and is said to be in the *relative refractory period*, or in a hyponormal condition. During this period the nerve recovers its power to respond to ordinary stimulation. Following the *hyponormal phase* there is a period when the nerve is *hypernormal*.

For much information in regard to nerve action, the world is indebted to the English physiologists, among whom Keith Lucas and Adrian take high rank as investigators. Lucas attempted to determine whether the nervous impulse is a variable quantity or whether it is always of the same strength in each unit fiber. He calls attention to the fact that it cannot be inferred with certainty that the magnitude of a contraction of a muscle is determined by the intensity of the stimulus given by the individual motor nerve fibers. The strength of the contraction may as readily be influenced by a variation in the number of fibers involved, as by a variation in the strength of stimulus over a constant number of fibers; he also calls attention to the fact that a study of the electrical changes taking place in the nerve fiber is no more satisfactory as a means of answering this question.

Lucas has reviewed the experiments of a number of investigators who have made use of the *gas-chamber experiment* upon nerve. In these experiments the nerve of a nerve-muscle preparation is passed through a chamber or chambers of glass through which carbon dioxide, alcohol vapor, or other gas may be passed at will. These chambers may be made of varying sizes, so that the length of the fiber exposed to the narcotic may be modified (Fig. 101). The first general result of this work shows that when a stimulus is applied to a nerve in such a way that the impulse must pass through a portion which has been narcotized by some gas passed through the chamber, the impulse becomes gradually less intense,

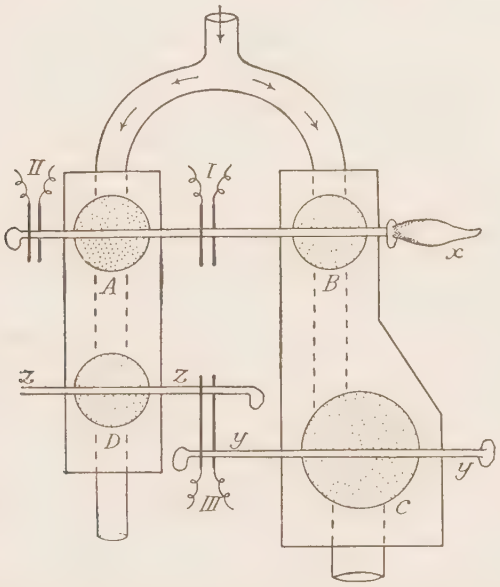


FIG. 101.—Diagram of apparatus used by Adrian in gas-chamber experiment. *A* and *B* are chambers, each 4.5 mm. in diameter. *C* is a chamber 9 mm. in diameter. I, II, and III are electrodes for stimulating the nerves of the nerve-muscle preparations *x*, *y*, and *z* respectively. The nerves are passed through the chambers and sealed in place with vaseline. Alcohol vapor or other gas is passed through the system in the direction of the arrows. The time required to produce a depth of narcosis sufficient to prevent the transmission of an impulse through the nerve, so as to cause contraction of the muscle, is measured.

and if the narcotized portion is long enough, the impulse fails entirely to bring about a muscular contraction. Others have found that there is a very definite relation between the length of fiber exposed and the depth of narcosis required completely to abolish the conduction of the nerve impulse. The longer the portion of nerve narcotized the less the depth of narcosis needed. From this fact came the suggestion that the nerve impulse suffers a *continuous decrement* in intensity rather than sudden extinction, as it proceeds along narcotized nerve. This suggestion at once raises the question as to the applicability of the *all-or-nothing law* to nerve action in normal nerve.

The All-or-nothing Law.—By means of the gas-chamber experiment, Adrian has been able to show that when a portion of a nerve has been narcotized just enough to prevent the conduction of impulses aroused by stimuli adequate to bring about maximal contractions in the normal muscle-nerve preparation, the intensity of the stimulus may be increased many fold without causing any contraction. From this, Adrian concludes that the nerve impulse is either maximal or zero.

Adrian has also succeeded in showing that when a nerve impulse which has passed through a partially narcotized region, *i.e.*, a *region of decrement*, emerges into an unnarcotized region, it immediately regains its original maximal intensity.

These experiments may be considered to offer evidence in favor of the idea that, in any given normal fiber, nerve impulses (aroused by electrical stimulation) are of uniform intensity, no matter how great may be the intensity of the stimulus applied. Whether this means that nerve cells can under no conditions send out impulses of varying intensities seems not yet to have been proved.

The Refractory Period.—Attention has already been called to the refractory period or phase of heart muscle, *i.e.*, the period following stimulation in which no stimulus, however strong, is effective in bringing about a contraction. Adrian and Lucas have succeeded in showing that this refractory period is, in the nerve of the frog at 14.8°C. *absolute* for 0.0025 second. Following immediately after this is a period of hyponormal excitability lasting till 0.012 second after the stimulation, and then a period of hypernormal excitability which continues till about 0.028 second after stimulation, when the excitability of the nerve returns to normal. This third phase of the refractory state is apparently due to an excess of H' ions. The duration of the refractory state is subject to some modification. A stimulus applied during the hyponormal period of excitability following a previous stimulation leads to a shorter refractory period than normal. Lucas also found that the refractory condition is associated with the *propagated disturbance*, for a stimulus which occurs within the absolute refractory period of a previous stimulation does not prolong that refractory period, nor does it interfere in any way with the effectiveness of a third stimulation, which may come at an appropriate interval after the first. The absolute refractory period of the nerve seems to correspond in a way to the latent period of muscle; the relative refractory period, or period of hyponormal excitability, to the recovery phase of the muscle.

Temperature Coefficient of Refractory Period.—One of the most significant contributions to knowledge of nerve action came from Adrian's work upon the temperature coefficient of the refractory period in nerve. The rate of recovery of excitability was measured by determining the full recovery curve of a nerve preparation. It was found that a change

in temperature affects to the same extent all stages in the recovery process. The Q_{10} of the absolute refractory period is the same as the Q_{10} of the relative refractory period; and the Q_{10} of the rate of recovery of excitability is the same for all degrees of recovery. This suggests that a single process accounts for all the phenomena of the refractory state. Q_{10} for the interval 10° to 20° is 2.90; Q_{10} for the interval 8° to 18° is 2.97.

Adrian makes the statement that the rate of recovery is a logarithmic, and not a linear, function of temperature, but that it does not follow that the process of recovery is, of necessity, a chemical as distinct from a physical process.

The Local Excitatory Process.—In just what the local excitatory process consists is not known, though the investigations of Lucas, Hill, Lapicque, and others indicate that it is due to a concentration of ions at some point in the tissue.

The Propagated Disturbance.—Adrian's work, the account of which has been given above, is a sufficient indication of the fact that the nervous impulse is due to some change in the fiber itself. Alexander Forbes in reviewing Lucas' work, says:

> It [the nervous impulse] has been likened (by Lucas) to the burning of a train of gunpowder, in contrast with the transmission of a sound wave whose energy comes entirely from its initiating source. This fact, now well established, should put an end to all efforts to explain the nervous impulse simply as a transient current of electricity conducted along the fiber on the same principle as in an insulated wire; the dynamics of the two modes of conduction are fundamentally different. The changes occurring in the nerve fiber follow the all-or-nothing law, and are accompanied by electrical changes which are certain indicators of the functioning of the nerve elements. Such electrical changes—currents of action—also characterize the activities of muscle and gland tissues. By some authors these electrical changes have been looked upon as of fundamental importance. To the present author, they seem more likely to be incidental phenomena attendant upon other more fundamental changes, the nature of which may be yet hidden.

Alexander Forbes calls attention to the fact that the heart muscle, the individual skeletal muscle fiber, and the nerve fiber have certain features in common:

1. All give evidence of the all-or-nothing law.
2. All show the refractory phase; the period of recovery follows the same sort of course in each case.
3. The duration of the refractory phase differs quantitatively in these tissues in a manner proportional to their excitation times.
4. Electric response occurs in all these tissues, has much the same form in each, and its total duration, rate of increase to maximum, and rate of decline bear nearly the same relation in the three tissues as do their refractory phases and excitation times.

From these facts Forbes concludes that the tissues mentioned show a community of functional tendency, and that the greatest difference between them is that of time relations. He also suggests that, although the all-or-nothing law, refractory phase, and action current have not been proved to hold in neuromuscular junction tissue, it is very likely, because of its excitatory properties, that these do exist, and that the duration of response and of the refractory phase will be found to occupy about one-third of the amount of time as do those of the motor nerves.

Rate of Conduction of Propagated Disturbance.—While it is usual to think of nerves as the chief conducting structures, the facts that conduction occurs in muscle as well, and that conduction is also involved in maintaining the proper correlation and coordination of ciliary activity must not be lost sight of. That there has been something of an evolution in the matter of nervous conduction is shown by a comparison of the rates of conduction in a number of different forms. Those forms which occupy the lower positions in the animal scale show, on the whole, the slower rates of conduction. The following figures indicate this general fact:

TABLE 52

Metridium	121–146 millimeters per second
Medusa	444 millimeters per second
Worms (Lumbriconereis)	420–2400 millimeters per second
Limulus	3.25 meters per second
Lobster	6–12 meters per second
Hagfish	4.5 meters per second
Snake	14 meters per second
Frog	28–30 meters per second
Man	125 meters per second

If the conduction rate of the human being were measured at the same temperature as that of the frog, there would be a much closer correspondence than appears in the figures given. The rate of the human nerve conduction is measured at 37.5°C., while the measurements on the frog are ordinarily made at about 18 to 20°C.

The Reflex.—Analysis of the functions of the spinal cords of vertebrates, and of the nervous systems of the invertebrates as well, lead to what is known as the *reflex*, as the simplest complete nervous reaction. In its simplest form a reflex is a reaction started by the environment acting as a stimulus to a certain receptor cell; this acts upon some neurone which passes on the state of excitation thus started in itself to other neurones by means of its connections with them. The excitation thus initiated and transmitted finally passes outward from the central nervous system through or along some *final pathway* (motor neurone), and reaches some effector cell, as a muscle fiber or a gland cell. The nerve structures along which these impulses travel together comprise the reflex arc. All of the arc except the motor neurone may be referred to as the *afferent arc*.

As this discussion continues, passing in review from the lower forms to the higher, it will appear that there is a gradual condensation, centralization, and cephalization of the nerve elements so as to form more and more highly organized central nervous systems. Apparently, the whole object of this condensation, centralization, and cephalization is to provide for the bringing of afferent and efferent neurones into more intimate relations with each other, and through the development of a highly intricate network of interlacing association neurones, or adjustors, to make possible reactions of the whole organism through impulses set up by various environmental influences in limited and localized areas.

The Reflex Mechanism.—Reviewing the animal series from the Porifera, in which the simplest structural relations exist, involving a nerve net, and passing to those in which definite synaptic systems are developed, it is found that the *reflex mechanism* becomes definitely established. At first it is extremely simple, and then becomes more and more complex in the higher forms of animals.

In the following tabular statement there is shown, in a very diagrammatic way, the cell relations existing in a number of animal groups. The symbol = indicates continuity through a nerve-net arrangement; the symbol | indicates contact; the symbol ⋛ indicates a synapse or a neuromuscular junction.

Porifera ..... Epithelium cell || muscle cell

Cœlenterata .....
- Sense cell = muscle cell
  or
- Sense cell = ganglion cell of nerve ring = muscle cell

Annelida (Earthworm) ..
- Sense cell = muscle cell
  or
- Sense cell = ganglion cell of ventral cord ⋛ motor cell ⋛ muscle
  or
- Sense cell = ganglion cell of ventral cord ⋛ motor cell || muscle

Mollusca (Cephalopod) ... Sense cell ⋛ muscle cell

Vertebrates .....
- Sense cell || sensory ganglion cell ⋛ motor cell ⋛ muscle
- Sense cell || sensory ganglion cell ⋛ adjustor ⋛ motor cell ⋛ muscle
- Sense cell || sensory ganglion cell ⋛ adjustor ⋛ adjustor ⋛ motor cell ⋛ muscle

These very diagrammatic schemes show the possibilities of:

1. A single sensory cell serving to throw into operation a single muscle fiber.

2. Two or more sensory fibers serving to throw into operation a single muscle fiber.

3. A single sensory cell serving to throw into operation a single muscle cell through a selection of one out of numerous pathways.

Conduction in Peripheral Nerve and in Reflex Arc Compared.—Among the physiologists who have contributed much to the understand-

ing of reflex action there may be mentioned Adrian, Lucas, Sherrington, Forbes, Lillie, Wedensky, etc.

Attention has already been called to the rate at which a nerve impulse is conducted in a peripheral nerve, and to some of the other phenomena associated with the passage of the nervous impulse. It is now the purpose to note some of the particulars in which conduction in the reflex arc differs from that of the peripheral nerve. It is frankly acknowledged that the following statements are based largely upon the results of investigations made upon the nerves of higher animals. Relatively few experiments upon reflex action in the lower animals have come to the author's attention. It seems very likely that a careful comparative study would reveal some striking and illuminating differences of reaction.

1. The *rate of conduction* in the reflex arc is much slower than in the peripheral nerve. Forbes calls attention to the similarity shown by the delay here and the delay at the neuromuscular junction in the nerve-muscle preparation.

2. Reflex action involves the phenomenon known as *afterdischarge*. By this, it is meant that the discharge of the motor neurone may continue for an appreciable time, sometimes several seconds, after all the afferent impulses have ceased to enter the central nervous system. The length of the period of discharge seems to be proportional to the strength of the stimulus. In order to provide for the afterdischarge, it is only necessary to assume the existence of a sufficiently elaborate and extensive chain of neurones. In such a chain each synapse adds delay, and as impulses may, in a complicated system pass over a great variety of pathways in reaching a given point, they may come through to the common final path in a considerably extended series of impulses.

3. *Summation.*—It will be recalled that frequently, when a series of stimuli, any one of which is inadequate to produce a muscle contraction, are sent along the nerve of a nerve-muscle preparation, there will finally be induced a contraction of the muscle. This is spoken of as being due to the summation of inadequate stimuli. In reflex action there is a phenomenon which bears a superficial resemblance to that which occurs in the nerve-muscle preparation, but which is in reality quite different. In the nerve-muscle preparation, no single stimulus is effective in bringing about the change in the nerve fiber known as the local excitatory process, and without this there can be no propagated disturbance. In the reflex summation, each individual stimulus is effective in setting up a nerve impulse, but the single impulse is ineffective in bringing about a reflex—a series of impulses is required. This is called the *summation of propagated disturbances.* As an example of this summation of propagated disturbances, the experiment referred to by Bayliss may be cited, in which the scratch reflex could be elicited in the dog only

after forty-four shocks had been applied to the skin, at the rate of eighteen per second.

Adrian and Lucas have shown that this condition may exist in a nerve-muscle preparation which has been fatigued, when there is in the nerve-muscle junction a region of decrement sufficient to block a single impulse, and the impulses are so timed that the second impulse comes into the nerve during the hypernormal phase of excitability. Adrian has shown that this occurs only in an acid medium.

4. *Irreversibility.*—It has been shown repeatedly that conduction may take place in the nerve fiber in either direction. In the reflex arc the synapse acts as a sort of valve and allows conduction to take place only in one direction, from afferent or associational neuron to motor neuron. This irreversibility may be due to the fact that the synaptic membrane, lying between the terminal branches of the two neurones, is permeable to certain ions only in one direction.

R. S. Lillie has offered the suggestion that irreversibility may be associated with the relation between the action current, at any single point in the path of conduction, and the natural rhythmic period of the next point in the pathway. It has been pointed out that the nervous impulse is not to be considered as a single discharge from a nerve cell, but rather as a rhythmic series of discharges, following each other in a regular and definitely timed order. It is probably true that the rate of the rhythm is different in different nerve fibers. In fact, cases are known in which the rates of the rhythms lie between forty and several hundred per second. Now, if it should happen that the rhythmic rate of the conducting pathways on the two sides of the synaptic membrane should be different, it would be easy to account for the fact that conduction can take place through the synapse in only one direction.

5. *Fatigue.*—It has long been accepted as a fact that nerve trunks are little subject to fatigue. This is in great contrast to the condition which exists in the reflex arc. Sherrington has shown, and Forbes concurs, that, in the reflex, fatigue occurs at the synapse antecedent to the motor neuron. The synapse, like the neuromuscular junction, appears to be a region in which conduction with decrement may occur. Fatigue appears here, as well as in the nerve-muscle preparation, to be associated with an acid condition; and as the acid condition becomes the more pronounced the degree of decrement and the amount of fatigue also increase.

6. *Variability of Threshold.*—It has been recognized by physiologists that in any individual motor nerve, or sensory nerve, there is not any very great variability in the threshold value, *i.e.*, the degree of stimulus required to bring about a response. In both afferent and efferent nerves the all-or-none law appears to hold. The threshold of the reflex, on the other hand, shows more variability than is found in either the afferent or the efferent nerves, or in the two summed together. In

discussing this point, Forbes refers to the experiments of Lutz, who showed that:

In the frog, with electrodes applied to an afferent nerve, the average threshold for the flexion reflex was twice as high as for the muscular response with electrodes similarly applied to the motor nerve. He further found that when the animal was cooled the threshold for the reflex rose nine times as much, per degree Centigrade, as did the threshold of the nerve-muscle preparation.

This may be explained if (as histological evidence certainly gives the right to do) it is assumed that any single afferent neuron is not limited in its synaptic relations to a single motor neuron, but rather that the afferent fibers, through their numerous branchings, may each be connected with many motor neurones, and *vice versa*. In such an arrangement as this, the excitation of many afferent neurones might have a very different effect, through the convergence of impulses, from that produced by the excitation of one or of only a few afferent fibers.

The reflex threshold would then depend on the number of afferent fibers excited, and no longer would be identical with the threshold of the most excitable fiber among them. In this way we could readily account for great variations in the reflex threshold through agencies which cause little change in the threshold of the afferent fibers.

Forbes directs attention to the fact that this convergence of impulses need not in any way enter into conflict with the all-or-nothing principle. In this convergence there need be no gradation of activity such as would be out of harmony with other facts known and generally accepted. Some portion of the central conducting path may be thought of as having a shorter refractory period than the afferent fibers. If there were a portion of the synaptic region which possesses a very brief refractory phase common to all the afferent paths involved, it would be possible to account for the effect of the converging impulses. If these converging impulses, set up by some single shock, travel over their varied pathways, they will come to the common pathway at slightly different times. A very brief refractory phase might make it possible for some impulses to arrive at the synapse during the hypernormal phase of recovery, and thus, in combination with preceding impulses, make it possible for them to break through a resistance which, without such combination, would have tended to extinguish them.

Forbes says:

We may conclude that the great variability of the apparent threshold of stimulation, in evoking reflex responses, points strongly to the central intermingling or convergence of conducting paths (evidenced histologically in the branching of fibers) as an essential feature in the interpretation of experimental results. We may further conclude that, though these experiments offer difficulties in the way of picturing central activity in terms of the properties of

functional response known in peripheral tissues, these difficulties may not be insuperable, possible ways of surmounting them being offered both by the character of decremental conduction and by great brevity of refractory phase somewhere in the central part of the path.

7. *Reinforcement and Inhibition.*—There are two mutual relations existing among reflex arcs which serve to distinguish conduction in the reflex arc from that of the peripheral nerve. These are *reinforcement* and *inhibition.* The terms *reinforcement, facilitation,* and *Bahnung* refer to conditions in which the activity of some reflex arc is reinforced by the stimulation of an allied arc. The phenomenon appears to be due to the convergence of conducting pathways from various points of external origin to common points in the central system.

The exactly opposite effect is that phenomenon known as *reflex inhibition.* An inhibition is that process by which an impulse is hindered or prevented by some factor applied through another impulse.

Inhibitions are of two general types, peripheral and central.

a. Peripheral Inhibitions.—These include those inhibitions which occur in certain skeletal muscles, especially among the invertebrates. In the nerve of the claw of the crayfish there are, for example, fibers which divide into two axis cylinders. One of these axis cylinders passes to the muscle closing the pincer of the claw, and the other to the muscle which opens the pincer. One branch is excitatory in action and the other is inhibitory. The particular function played by the branch of the nerve is determined by the character of the end organ in which it terminates. Inhibitions of the peripheral type are, among vertebrates, limited to involuntary muscle.

b. Central Inhibitions.—These are inhibitions mediated through the central nervous system or through various scattered ganglia. Inhibitions which occur in the reflex control of respiratory movements, of peristalsis, of the reciprocal inhibitions of skeletal muscles, among the vertebrates, etc., may be mentioned as examples.

Reciprocal Inhibition of Antagonistic Muscles.—One of the most interesting cases of inhibition is that which has been brought to light through the investigations of Sherrington. Many of the skeletal muscles of the vertebrates are arranged in opposing groups. Thus, there are flexor muscles which are opposed in their action to extensor muscles. While it is necessary that some fibers of such opposing muscle groups be stimulated at the same time, as in maintaining body posture, standing, etc., it is necessary that some means be found to prevent these opposing muscles from antagonizing each other at other times and under other conditions. No one has succeeded in demonstrating the existence of efferent fibers which, upon stimulation, cause skeletal muscles to relax. Relaxation must come as the result of the inhibition of the motor nerve cells which, when active, cause the muscle fibers to contract. The real

control is in the central nervous system. Sherrington points out that, in the mammal, stimulation of an afferent nerve in a hind limb leads normally to the contraction of the flexor muscles of that limb, and to the contraction of the extensor muscles of the opposite limb. In each case there is the inhibition of the opposing group of muscles.

A very striking example of reflex inhibition is that shown by the decerebrate mammal (cat). Let the motor nerves leading to all of the muscles acting upon the knee joint of the hind leg, except the extensor (vasto crureus), be cut. This results in the paralysis of all except the extensor muscle. The animal is now placed upon its back with its partially paralyzed leg held in a clamp in a vertical position. Stimulation of the nerve leading to the extensor muscle raises the foot. Relaxation of the extensor is shown by the fall of the foot. In many cases the decerebrate mammal shows *decerebrate rigidity*, that is, a sustained tonic contraction of the extensor muscle. In case the muscle does not show this tonic contraction, it can be made to do so by stimulating the skin of the opposite hind leg. This is known as the *crossed extension reflex*. If, while the muscle is strongly contracted, an afferent nerve in the same leg is stimulated, there will be an immediate relaxation of the extensor muscle and the foot will fall. Forbes says:

> This relaxation is a part of the flexion reflex which is the obvious, defensive reaction whereby an animal in normal life withdraws its foot from a thorn or other object which causes injury. Here we have a case in which the stimulation of an afferent nerve leads to an absence of activity on the part of certain motor cells.

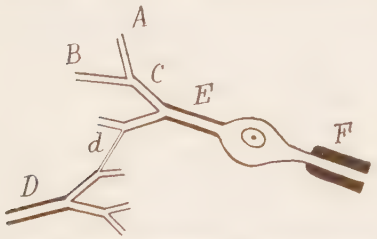


FIG. 102.—Diagram of motor neurone with hypothetical arrangement of conducting paths. (*According to Alexander Forbes.*) (For description see text.)

As to the mechanism which is involved in such inhibitions, knowledge is wanting. Forbes has given a suggestion which, while only a suggestion, may have in it enough of value to warrant consideration. He postulates such a condition as is represented in Fig. 102, in which there is an arrangement of branches providing for reinforcement by convergence of impulses at one point, and for inhibition by impulses approaching from another source. In this figure

A and *B* are pathways whose impulses reinforce each other on the principle of summation.

C—*A* is a region of decrement with very brief refractory phase, such that a single impulse fails to pass. If impulses from *A* and *B* arrive at the right intervals, summation may occur and a second impulse falling in the hypernormal phase will pass the remainder of the dendrite, and finally set up an impulse in *F*.

D—*A* is a path through which inhibitory impulses arrive. Any impulse arriving through *D*, when passing through the most attenuated portion *d*, will be broken up into a series of impulses of so high frequency that each reaches the large main trunk of *E* early in its hyponormal phase. The disturbance in *E* is, therefore, subnormal and fails to pass the region of decrement *E*. So long as impulses approach through *D*, *E* is the seat of a total block to impulses coming to *E* from all branches of the dendrite.

E—*A* is a region of decrement, and the seat of a total block to impulses coming from all branches of the dendrite.

Dr. Forbes has also worked out a purely hypothetical scheme for the organization of the spinal centers, involving the substance of the scheme just presented above and providing for most, if not all, of the known facts involved in reflexes and inhibitions. He dispenses with the assumption of convergence as the basis of inhibitory frequency in the extensor centers,

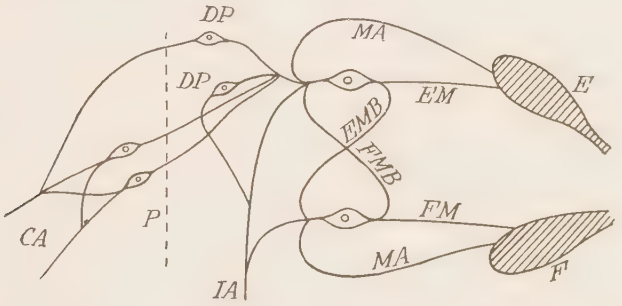


FIG. 103.—Diagram to illustrate Forbes' simplified scheme of spinal neurones: *E*, extensor muscle; *F*, flexor muscle; *P*, median plane; *CA*, contralateral afferent fibers; *IA*, ipselateral afferent fibers; *DP*, delay paths, representing extensive central connections to provide for prolonged after-discharge; *MA*, afferent neurone from muscle; *EM*, extensor motor neurones; *FM*, flexor motor neurones; *EMB*, collateral branch of extensor motor neurone; *FMB*, collateral branch of flexor motor neurone.

and refers this frequency to the breaking up of a single impulse into a series of briefer ones in the finer branches.

Forbes' scheme, pictured in Fig. 103, represents two muscles of the same limb, an extensor *E* and a flexor *F*, together with their nervous connections. A typical afferent nerve *IA* enters the spinal cord and divides into three branches, one branch going to the flexor motor cell, one to the extensor motor cell, and one to the delay path. From each motor cell a motor fiber passes to its appropriate muscle, and the activity of the cell would always tend to throw the corresponding muscle into action except for the provision made for inhibition. From the axis cylinder of each motor fiber a collateral branch is given off soon after the fiber leaves the cell body. This branch passes into synaptic relations with the opposing motor cell, and under the appropriate conditions tends to inhibit the action of

that cell. If the synaptic relations are of the type shown in Fig. 102*d*, reciprocal inhibition of antagonistic muscles becomes understandable.

It should also be noted that the afferent paths *MA* leading from the muscle to its appropriate motor cell provide for the passage of impulses from the muscle to the motor cell. Such impulses tend reflexly further to stimulate the muscle to action.

Excitation of the extensor center is largely dependent upon the summation effect of convergence from different lines. Excitation of the flexors, on the other hand, appears to be (at least in many cases) the dominant reaction. It is assumed, therefore, that the approaches to the flexor motor neurone are of such a sort that even if all the afferent fibers in the nerve are excited they will not set up inhibitions in the flexor motor neurone. The approaches to the extensor motor neurone, on the other hand, are more numerous and of a sort to bring about an inhibitory frequency in this neurone, provided that all, or most, of the afferent fibers are stimulated, and thus an inhibition of the extensor muscle, no matter how many impulses may be arriving from other sources.

Impulses passing to the motor cells through the delay paths are held to account for the phenomena of afterdischarge.

Types of Nervous Organization.—It is, of course, beyond the scope of this work to study in detail the structure of any large number of animal forms. The most we may hope to do is to acquire some information as to the general plan of structure or architecture of the nervous systems of a few types of animals, and, thus, possibly gain some understanding of the way in which the nervous mechanism may operate. The diagrams present, in about their lowest terms, ideas as to the general arrangement of parts in the animals mentioned.

The Primitive Receptor-effector Mechanism of Sponges.—Parker has shown that there is no true nervous system among the sponges. The cells about the oscula are slightly contractile, and may be directly stimulated in a variety of ways. When these cells are stimulated, they behave in very much the same way as smooth muscle, except that they respond more slowly, finally closing the osculum so that the water currents are slowed up or stopped. There is also, probably, a very slow transmission of a neuroid character of the impulse from one part of the body to another. This transmission can hardly be looked upon as a sign of the existence of true nerve elements among the sponges, but rather as an expression of the general property of conductivity, common to all protoplasm.

Parker says:

> Sponges, then, represent that stage of evolution in which a primitive type of muscle tissue has made its appearance unaccompanied with nervous elements. . . . Sponges may be said to have among their cell combinations effectors, but

no receptors or adjustors. They mark the beginnings of the neuromuscular mechanism in that they possess the original and most ancient of its constituents, muscle, round which the remainder of the system is supposed subsequently to have been evolved.

Parker has also called attention to the position of the nucleated portion of the neurone in neurones of different types and in different animals. The primitive protoneurone (motor) of the Cœlenterate nerve net has its cell body centrally located. In the nerve nets in which polarization is exhibited, the cell bodies are found nearer the receptive than the discharge end. This is the condition found in the central nervous organs of the worms, Arthropods, and Molluscs (Fig. 104).

A second type of migration occurs in the sense cells of the nerve nets and in those cells that are derived from them and that are associated with the more differentiated sensory surfaces of the higher animals (Fig. 105 (Parker)). In the Cœlenterates the sense cells or receptor protoneurone

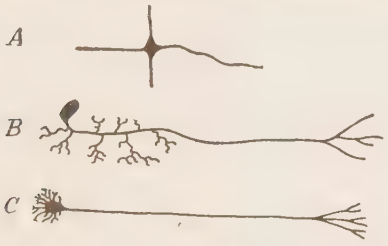


Fig. 104.

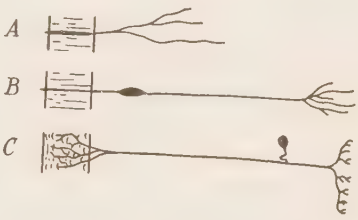


Fig. 105.

Fig. 104.—Motor nerve cells: *A*, motor cells from nerve net of Coelenterate; *B*, motor neurone from earthworm; *C*, motor neurone from vertebrate. In *B* and *C* the central end of the neurone is toward the left. (*After Parker.*)

Fig. 105.—Sensory nerve cells: *A*, sensory nerve cell of Coelenterate; *B*, sensory neurone of Mollusc; *C*, sensory neurone of vertebrate. In each case the peripheral end of the cell is toward the left. (*After Parker.*)

has its cell body at the receptive end, whence its fibrous prolongation reaches into the nerve net. Much the same condition occurs in the earthworm, though in many other worms the cell body has moved inward, leaving only a cell process in connection with the sensory surface. Among the vertebrates the migration has continued to such an extent that the cell body of the primary receptive neurone has come to lie much nearer to the central organ than to the peripheral surface from which it started. These two types of migration of peripheral and of central cells may be expressed thus:

Primary sensory neurone: Cell body migrates away from receptive pole.

Central neurone: Cell body migrates toward receptive pole.

Parker has also called attention to the probability that these migrations, involving, as they do, the trophic or nutritive centers of the cells, are to be accounted for by the changed metabolic needs of the evolving

neurones. As an example, Parker cites the conditions presented by the receptive neurones of chemical sense among the vertebrates. He finds:

1. Olfactory neurones (Fig. 106*A*) in which the trophic center is at the receptive end of the cell, a condition equivalent to that found in sea anemones and earthworms.

2. Chemical-sense neurones (Fig. 106*B*), in which the trophic center of the receptive cell has migrated far inward toward the central organ. This condition is found among vertebrates.

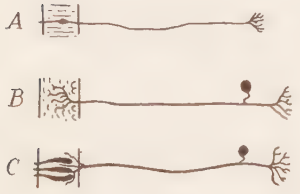


FIG. 106.—Primary neurones of the three chemical senses of vertebrates: *A*, olfactory neurone; *B*, common chemical sense; *C*, gustatory sense. In each case the peripheral end of neurone is toward the left. (*After Parker.*)

3. Gustatory neurones (Fig. 106*C*), like those of group 2, except that certain integumentary cells have been appropriated to serve as secondary sense cells. Such are the taste buds of the mammals.

When the trophic center is at the receptive end of the neurone, that end of the neurone is thousands of times more sensitive than when the center has migrated far from it. This loss in sensitivity is in part compensated for through the appropriation of the secondary cells, whose trophic centers may then, in part, take the place of those which have migrated inward.

Nervous Mechanisms of the Cœlenterata.—The nervous system in the Cœlenterata takes on the form of a diffuse network. Reference has already been made to the diffuse character of the nerve net in Hydra. Similar conditions exist in many forms, some of which reach considerable size, *e.g.*, the Actinians. This network is made up of protoneurones and these have nerve fibers which branch and anastomose in a very intricate manner. The network is in many forms scattered diffusely beneath the epithelium over the whole surface of the body. In the less specialized forms the protoneurones are in physiological connection with the motor cells, but differentiated sensory or receptor cells do not occur. Stimulation at any part of the net spreads with some rapidity throughout the nerve net and results in the activation of the motor cells. In other forms there are definite receptor structures which are in very direct communication with ganglion cells, and through these with the motor cells. In Fig. 107, a diagrammatic scheme of a simple

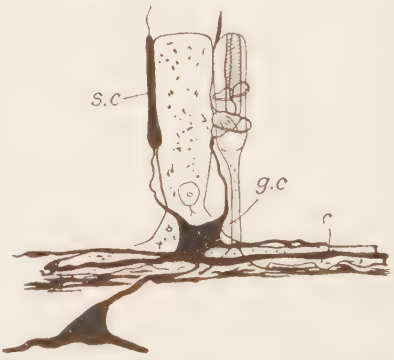


FIG. 107.—Diagram of the intercellular reflex arc in hydroid polyp: *s.c.*, sensory cell; *g.c.*, ganglion cell; *c*, contractile fiber. (*Ac. to Wolff, from Pütter.*)

reflex arc is shown as it occurs in some of the Cœlenterata. This figure, taken from Pütter, shows a sense cell of the surface epithelium connecting directly with a ganglion cell, and this, in turn, with its effector, a motor cell. In some of the higher forms, a tendency of the nerve net to become somewhat concentrated and centralized is noted. For example, among the Craspedote medusæ a nerve ring, made up of cells and fibers is found about the margin of the bell, in close association with the marginal sense

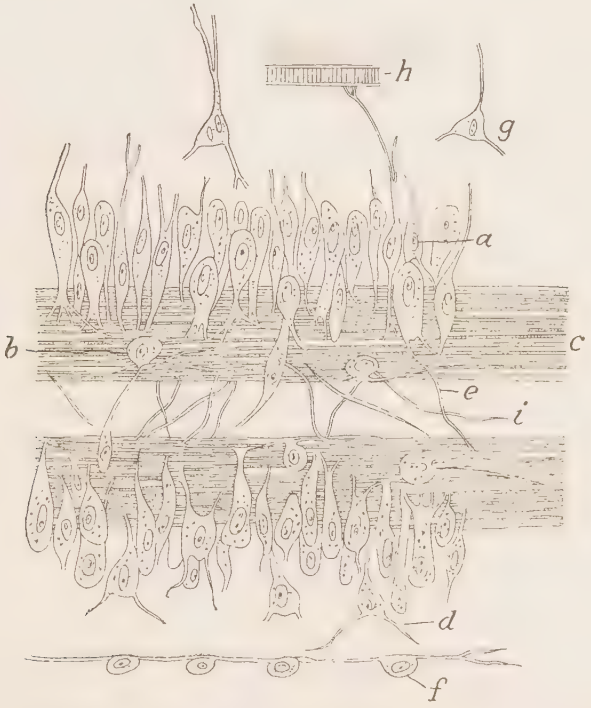


FIG. 108.—Schematic view of a piece of the marginal nerve ring of *Gonionemus murbachii: a*, cells placed transversely to the upper nerve ring; *b*, ovoid cells with sheathed fibers; *c*, nerve fibers parallel to the marginal ring; *d*, multipolar cells proximal to lower ring cells; *e*, communicating fibers between rings; *f*, ovoid cells in marginal strand; *g*, multipolar cell of the subepithelial network; *h*, muscle fiber; *i*, space between upper and lower ring. (*After Ida M. Hyde.*)

organs and with the muscles by which the rhythmic swimming movements are effected. There is a strong tendency, in those forms which have radial gastric canals and pendulous manubria, for the nerve net to be somewhat emphasized in these regions.

As an example of the nervous organization among the higher Cœlenterates, the case of the medusa Gonionemus may be cited. This medusa is one found in some abundance at Woods Hole, at Vineyard Haven, and some other points along the Atlantic coast. This animal is an umbrella-shaped form with a manubrium occupying the place of the handle of the

umbrella. About the margin of the umbrella (Fig. 108) are two well-defined bands of nervous tissue consisting of both cells and fibers, and a less well-defined band occupying a position peripheral to the two main bands. Along the radial canals (Fig. 109) extensions of nervous tissue also appear, both cells and fibers. The arrangement of cells and fibers is not quite that of a typical nerve net, nor is it that of the more centralized systems appearing in worms, Crustacea, and higher forms. It will be recalled that in the medusa locomotion is accomplished by contractions of the muscles of the velum, which partially closes the opening of the umbrella, and by contraction of muscles in the margin of the bell itself. One of the two broad bands lies just above, and the other just below, the attachment of the velum to the bell. Each band is a circular

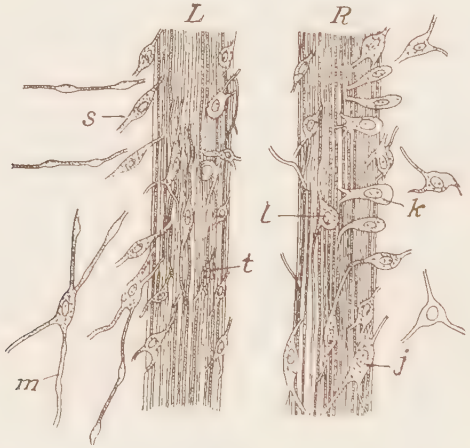


FIG. 109.—Schematic view of a piece of radial nerve tissue of *Gonionemus murbachii*. The band at the right (*R*) represents the nerve cells below the ectodermal epithelium of the subumbrella; at the left (*L*) those between the muscles and the endodermal cells as seen from the exumbrellar side: *j*, multipolar cells, close to radial nerve fibers; *k*, radial tissue cells; *l*, ovoid cells among the radial nerve fibers; *m*, beaded fiber of subepithelial network; *s*, bipolar cells; *t*, multipolar cells. (*After Ida M. Hyde.*)

ring composed of fibers extending about the margin of the bell. Attached to the fibers are bipolar and multipolar nerve cells which lie transversely to the fibers of the rings. It is possible to see fibers extending from one band to the other, and there may be anastomoses between the fibers from different cells, though this condition has not been demonstrated so far as the author is aware. Fibers pass from these rings to the musculature of the body and to the rings from the marginal sense organs. Evidence of the importance of the marginal sense organs appears in the fact that, when the medusa is deprived of all its so-called eye spots, normal rhythmic activity ceases, but so long as a single eye spot remains functional, normal rhythmic contractions continue.

Rhythmical Pulsations of Medusæ.—A. G. Mayer studied rhythmical pulsation in certain marine forms such as the medusæ Cassiopea, Aurelia, and Dactylometra, the arms of the barnacle Lepas, and the hearts of Salpa and of the loggerhead turtle. As is well known, these structures carry on normally a definite and regular rhythmic movement. Mayer found in the medusa Cassiopea, as has been stated above for Gonionemus, that cutting off the marginal sense organs paralyzes the disc so that it does not continue to beat rhythmically. The disc from which the marginal sense organs have been removed can, however, be made to pulsate if it is cut so as to form a ring, or if a series of concentric, broken-ring-like cuts be made through the muscular tissue of the subumbrella. (Diagrams of the cuts made can be found in *Publication* 47 of the Carnegie Institution of Washington.) Any momentary stimulus of the disc will usually result in rapid, rhythmical pulsations, which may be long continued.

The waves of pulsation all arise from a common stimulated point, and the tissue must form a completely closed circuit. In such a ring as has been described, the contraction must start in *one* direction through the circuit. When this occurs, and the ring is large enough to allow each point in the ring a period of rest before the return of the stimulating wave through the circuit, the wave of contraction will maintain itself for an indefinite period. If contraction waves of equal intensity start in opposite directions around the ring from the point of stimulation, they will meet on the opposite side of the ring and tend to neutralize each other. Each point in the path takes its share of responsibility for passing the wave to the next succeeding points. The stimulus to pulsation is carried by the nerve net, which is distributed diffusely in the subumbrella. As each wave comes to the point of origin, it is reinforced and is again sent out as a contraction wave. The rate of pulsation in the disc deprived of marginal sense organs is determined by the length of the course which the wave must run before it returns to its point of origin, and is always more rapid than in medusæ possessing the marginal sense organs. The rhythmic movements of the jellyfish arise, therefore, in definite points; the eye spots in the uninjured animals, and local excited centers in the demarginated animals.

The stimulus which causes the contraction wave is nervous in origin, and is transmitted through the subepidermal nerve net. The stimulus apparently arises as the result of the continuous formation of sodium oxalate in the marginal sense organs. This sodium oxalate precipitates calcium as calcium oxalate and sets free sodium chloride and sodium sulphate. These serve as nerve stimulants. The pulsation is then due to a slight excess of Na' ions maintained in the nervous centers in the sense organs over and above that found in the sea water.

Rate of Transmission in Metridium.—Parker has made an investigation of the rate of transmission of the nervous impulse in the nerve net of *Metridium marginatum*. This Cœlenterate is large enough to permit the preparation of considerable lengths of tissue through which the transmission may be accomplished. He found it possible to separate a narrow tongue of tissue along the edge of the foot for nearly one-half the perimeter of the animal, as shown in Fig. 110. By means of this preparation it was possible to measure transmission in the same way as nerve transmission is measured. Parker found that in this form the rate of transmission at 21°C. varies from 121 to 146 millimeters per second.

FIG. 110.—Diagram of preparation of *Metridium* by means of which Parker measured rate of transmission in the nerve-net. *x*, point of application of stimulus.

Nervous Systems of the Echinodermata and Their Actions.—The Echinodermata, and particularly the starfish, have served for the study of many objective problems involved in animal behavior. In reviewing the literature of this portion of the subject one is struck by the fact that some of the students in this field are either lacking in an understanding of the type of nervous structure which exists in these organisms and, therefore, of the types of response which may be expected to result from stimulation, or that they have had little interest in matters of pure physiology.

Among the Echinodermata generally, the nervous system is in the form of a nerve net or nerve plexus. Whether the nerve elements are of the type of the protoneurones which compose the nerve nets of the Cœlenterata or of a somewhat more highly differentiated type seems to be difficult to settle by microscopic examination. For the present, the term nerve net will be as applicable to the structures under discussion, although some of the physiological observations may raise question as to the desirability of the term. In these forms there is an aggregation of nerve elements in such a way as to form a central system, in addition to one or more less definitely aggregated arrangements of nerve elements in regions of the body somewhat remote from the central system. A clearer idea of existing conditions may perhaps be obtained from a description of the nervous systems found in the starfish. In the starfish there is a three-parted nerve system, each part working somewhat independently of the others.

1. *The Ectoneural System.*—This is a concentration of the elements of the nerve net in such a way as to form a ring (circumoral nerve ring) about the mouth, and five radial nerves extending outward from the circumoral nerve ring along the oral surface of the main radii of the animal. Fibers pass from the ring to the skin of the mouth and to the gastric wall. Nerves pass from the main radial nerve strands to all

the tube feet, pedicellariæ, eyes, respiratory papillæ, and other organs of the arms. Ganglion cells, sometimes bipolar, lie along the course of the fibers in these strands.

Local condensations of nervous tissue occur about the bases of the spines and of the pedicellariæ. While the existence of this condensation of elements is to be recognized, the fact must not be lost sight of that there is here, as in the Cœlenterata, a diffuse network of nerve elements extending over practically the whole surface of the body. The nerve net is a more elaborate and more complicated structure than appears among the Cœlenterata. Through the circumoral nerve ring, the radial nerve strands, and the diffuse nerve net, the coordination of body movement and that of the tube feet movements are brought about.

2. *The Hyponeural System.*—This is a nerve net found in the wall of the pseudhæmal canals, extending outward along the rays of the starfish. This system innervates some of the muscles of the body wall with its side branches. Here and there, the ganglion cells of this system are somewhat bunched together, and at the end of the rays definite ganglia are formed underneath each eye spot.

3. *The Entoneural System.*—This system lies in the peritoneal epithelium under the dorsal musculature of the rays and central disc, and is much less highly developed in the starfish than the other systems.

Receptors of various sorts occur in the superficial epithelium of the body. The animals are very sensitive to contact, and, as has been shown by Hamann and others, there are sensory nerve cells to which have been assigned the functions of chemical sense and light perception.

The starfish behaves normally as an individual only when the nervous system as a whole is in an uninjured condition. Evidence of this fact will appear in a later paragraph. While this is true, it is also true that much independence of action inheres in the different parts of the Echinoderm. For example, each ray of a starfish behaves as a unit if the nerve net be severed between it and each of the adjacent rays. The whole ray responds in a reflex manner to the stimuli coming to it.

Unfortunately, definite information is lacking as to the exact nature of the reflex arcs which must comprise a large part of the nervous system. It is possible, however, to derive from accounts which have been given of the behavior of the starfish certain facts as to the nature of the nervous mechanism and as to its physiological properties.

1. The surface of the body is provided with very numerous receptors of various sorts. Every respiratory papilla is very sensitive to *contact*. The animals also give evidence that they are sensitive to *light*, to *chemicals*, such as acids, alkalies, crystals of sea salt, potassium bichromate, and various food substances.

When the receptors on the aboral surface of the body are stimulated by an agent of the appropriate kind and intensity, a variety of responses

occurs. The first point to be noted here is the fact that there is a definite threshold of stimulation. There is an intensity of mechanical stimulation below which even repeated stimuli are ineffective. When the stimulus is adequate, some of the following activities may be noted:

a. The respiratory papillæ are withdrawn.

b. The rings of pedicellariæ about the bases of the spines, in the vicinity of the disturbed region, rise so that the spine is hidden. Each of the rings is provided with large numbers of pincer-like *pedicellariæ*, which are ready to act as weapons of offense or defense.

c. The spines all bend in toward the center of disturbance. There is, therefore, *conduction* of impulses. This conduction is of a diffuse nature, tending to spread in all directions from the point of stimulation.

d. The degree to which the reaction spreads depends upon the strength of the stimulus, the kind of stimulus, the part of the body stimulated, the age of the individual, and individual differences. It is found that, in general, older animals respond to stimulation more readily than younger ones, that a stimulus applied in the region of an eye spot is more effective than in some other regions, and that certain individuals are much more susceptible to stimulation than others. In some cases a very slight stimulus is sufficient to bring about a very marked reaction.

e. Summation of stimuli. It frequently occurs that a single shock is not sufficient to bring about the attack of the pedicellariæ. A repetition of the stimulus is required. It is possible that there is here a type of behavior akin to that of pigment cells or smooth muscle cells of the higher animals?

f. The response to the stimulus is not of a stereotyped sort. This may be taken as indicative of the fact that there are many pathways over which impulses may travel, even in so humble an animal as a starfish. It may also be possible that so simple an animal as the starfish may have some power to determine over which one of a possible series of pathways a given series of impulses is to travel. If this is true, the animal may be considered to have some choice in the type of reaction shown by its response to stimulation.

g. There is a period of *afterdischarge* in the system of reflex arcs of such a sort that, even after the localized stimulus has been removed, the response continues for some time.

h. After a response to a stimulus has started, it may be modified in its details by the application of some new stimulus, without altering the general trend of the reaction.

i. Evidence of that type of reaction which is called, in the higher animals, *reciprocal inhibition* is seen at times, especially in the behavior of the tube feet.

j. Jennings has called attention to the fact that chemical stimulation of a rosette of pedicellariæ renders it more susceptible to mechanical

stimulation later on. Also, mechanical stimulation leaves them, after subsidence, in such a state that they react more readily to stimuli from a distant region than do even rosettes nearer to the point of stimulation. Can this mean that the nervous tissue of the starfish has a refractory phase and that the second stimulation may possibly occur during the hypernormal phase? The reaction time is undoubtedly slower than in the reflexes of higher animals, the responses may be less exact, but they appear to be of the same general sort as the reflexes common to higher forms.

A. R. Moore has made a study of the normal righting reactions of the starfishes *Asterina miniata* and *Asterias ochracea*. He has found that there is a common or normal way in which righting takes place. Moore says:

For example, in Fig. 111 any two arms, say *A* and *B*, face each other ventrally and attach; *C* and *E* may have obtained a hold, but soon withdraw their tube feet, rise orally, pulling *D* with them, and complete the somersault. During

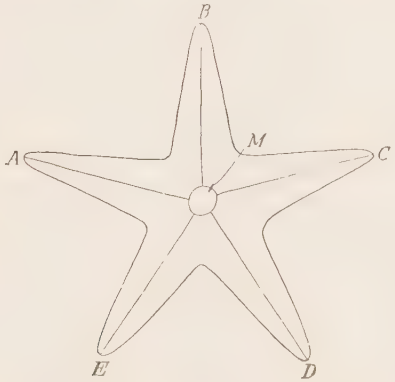


FIG. 111.—Diagram to illustrate arrangement of main portion of ectoneural nervous system of starfish: *A*, *B*, *C*, *D*, *E*, rays of star; *M*, position of cut in Moore's experiment. (*After Moore.*)

this process *D* may either remain passive or hold to the bottom with its tube feet and so retard the righting. Taking Loeb's view that *C* and *E* have their attaching inhibited by impulses sent from *A* and *B* and move upward at once, it is significant that *D* is affected by such impulses very slightly, if at all. Repeated observations of the passiveness of *D* led me to think that the inhibiting impulses arising from *A* and *B* lose so much in effectiveness, as they travel away from the point of origin, that they affect only adjacent arms sufficiently to cause movement. That is to say, an inhibiting impulse from *A* rouses only *E*, and the impulse from *B* causes only *C* to act. If such were the case, we should have additional proof that the hypothesis of Loeb is correct and that the righting of the starfish is accomplished by the simplest sort of mechanism.

This rapid decrease in the effectiveness of a nerve impulse, as it travels away from its point of origin, is a phenomenon familiar to physiologists in the spreading of impulses and was described by Pflüger and later by Sherrington with

reference to vertebrates. According to these authors, "the degree of reflex spinal intimacy between afferent and efferent spinal roots varies directly as their segmental proximity."

In order to test whether this were also true of the nervous system of the starfish, I cut the oral nerve ring of one of these animals between the arms *B* and *C* at *M* (Fig. 112). In the case of a starfish so treated, if *A* and *B* face each other ventrally and attach, *E* is inhibited; but *C* either remains passive or attaches and holds to the bottom until pulled loose by *E* and *D*. In addition to *A* and *B* attaching coordinately, *C* and *D* may also attach and face each other ventrally. In this case the starfish remains for some time in the position shown in Fig. 112, until, finally, *E* pulls either *A* or *D* loose and thus decides the course of righting. The time required for the righting to take place in this way is two or three times as great as that needed before the cut was made. Of course, if *B* and *C* should attach and inhibit *A* and *D*, then the righting would be accomplished as

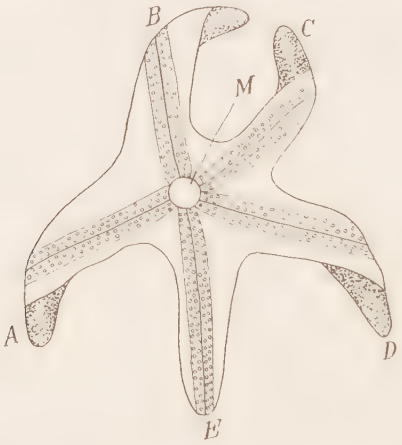


FIG. 112.—Moore's diagram showing attempt at righting after cut is made at *M*.

in the normal starfish. Likewise, if *A* and *E* or *E* and *D* attach coordinately, their connection with the adjacent arms is unimpaired and the righting is performed in the normal way. But when the ring nerve is cut at *M*, if *A* and *B* attach coordinately, *C* is inhibited very slightly or not at all and does not rise orally to assist in the righting as in the case of the uninjured animal; on the contrary, *C* acts as an independent arm and renders no assistance by coordinated movement.

In making the one cut in the ring of the starfish, none of the arms of the animal is isolated nervously; all are connected; but, instead of the old circular connection, there now obtains a linear system comparable to the nerve cord of worms and crustacea. If conscious judgment ever were present in the starfish, it scarcely could have been destroyed by the operation, because each part of the animal still has perfect nervous connection with every other part. The fact that the arm *C* does not coordinate with the active arms proves that direct nervous connection across the cut is necessary for coordination; that *C* can only be caused to move "intelligently" by an impulse from an adjacent arm.

This is different from saying that the coordinated movements of the starfish are due to judgment exercised by a psychoid entity. Hence, it is evident that there is no nervous center in the starfish, for any one of the five arms may give rise to impulses which strongly affect only adjacent arms. These impulses rapidly diminish in strength as they travel away from their point of origin, and their effect is usually imperceptible in the movements of other than adjacent arms. The simple nervous mechanism here described is sufficient to account for the coordinated righting movements of the starfish.

The nervous system of *Antedon rosaceus* is a very sensitive structure. Any strong stimulus will cause a fixed animal to detach itself and swim through the water until it finds some other object to which it may become attached. The swimming movements are brought about by the alternate flexions and extensions and aboral strokes of the arms. Each ray is composed of two arms, and these two arms are so coordinated that, during motor activity, while one arm is flexed the other is in extension. Moore has shown that there is here a case of reciprocal inhibition, similar to that which exists in the starfish, and that the mechanism may be thrown out of operation by subjecting the animals to poisoning by strychnine in a 1 to 10,000 solution for 10 minutes.

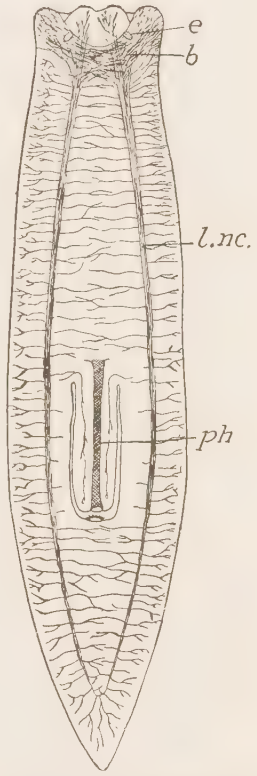


FIG. 113.—Diagrammatic representation of the nervous system of a Planarian worm: *e*, eye-spot; *b*, region of cerebral ganglion or brain; *l. nc.*, lateral nerve cord; *ph.*, pharynx.

Nervous Systems of the Worms.—As a contrast to the conditions existing among the Cœlenterata and Echinodermata, among the lower forms a greater centralization of the nervous elements is found, and a more clearly marked differentiation of function than has heretofore appeared. A tendency of the ganglion cells to become aggregated into two ganglionic masses in the anterior portion of the animal is noted. From these ganglia tracts of fibers, frequently branching and anastomosing with one another, and containing ganglion cells, reach backward throughout the body. There is a real difference here between central system and peripheral fibers, and at the same time the nervous network is sufficiently diffuse so that small pieces of a Planarian worm exhibit reflexes and good progressive movements. Figure 113, redrawn from Jijima's paper in the *Zeitschrift für Wissenschaftlichen Zoölogie*, gives a somewhat diagrammatic indication of the manner in which the nerve elements are arranged in a Planarian worm.

Significance of Polarity in Nervous Organization.—Numerous investigators have called attention to the fact and significance of polarity in animal organization. This polarity has as its basic physiological feature a differential metabolic activity of various parts of the animal, as, for example, exterior as contrasted with interior; anterior as contrasted with posterior; dorsal as contrasted with ventral, etc. The differences in metabolic behavior may be either in degree or in kind, or both.

Polarity may show itself in the behavior of the organism as a whole, even though there be no apparent structural differences in the different body regions which would seem to account for the differences in behavior. This is certainly true among some of the protozoa, and even in some of the simplest of the metazoa. The existence of these differences of metabolic activity is demonstrated, for example, in the fact that one part of an animal may regenerate a lost portion more rapidly than some other part of the same animal, or in the fact that such a part as a Hydroid stem regenerates a polyp at what was the polyp end, and a stolon at what was the stolon end of the stem.

Polarity, that is, differential metabolic activity, involves differential irritability. Irritability is an expression of colloidal instability. So, polarity comes to be an expression of the difference in degree and in kind of the irritabilities of those colloidal complexes which are called cells. Among animals, even low down in the animal scale, the development of special structures called receptors is associated with this adjustment of instability. These are, in the lowest forms, of a very generalized type; but in the higher forms are adapted to receive impulses or stimuli of specific kinds. Animals higher up in the animal scale have a greater variety of receptors, sensitive to a greater variety of external stimuli, and also adapted to receive stimuli of less intensity. Animals in which there have come to be a great variety of highly sensitive receptors may be spoken of as having highly differentiated nervous systems.

It is interesting to note that these differentiations usually involve one end of the organism more than the other. There is a tendency toward an accumulation of receptors at the anterior end. In many of the worms there is a secondary accumulation or concentration of receptor elements in the posterior end also, and a minimum of metabolic activity in the midregion of the body. This arrangement naturally involves, also, a condensation of nervous elements, *cephalization*, in the anterior end of the animal body. Such condensations begin to appear among the flatworms. Aggregations of nerve elements, particularly of the motor elements in the midventral line of the body, also appear among the worms. These may be included under the general term *centralization*. Among the Polychætæ and Oligochætæ the centralization of nervous elements has become quite pronounced. In the earthworm, Lumbricus, the process of cephalization is not so marked as in the marine worm, Nereis, while the

ventral nerve cord is about as well developed in one form as in the other. That is, the anterior end of the earthworm is less dominant than is the anterior end of the seaworm, Nereis. This probably means that the anterior end of Lumbricus has not the same degree of metabolic activity as is to be found in the anterior end of Nereis. Associated with this difference of metabolic activity is the great difference in the degree to which the receptor systems are developed in the two types of worms. The following statement represents the existing conditions:

Earthworm. No special sense organs are developed, unless the prostomium be so considered. Tactile, chemical, and photic receptors, sometimes grouped together, are located in the skin, particularly of the head region.

Marine worm. Receptors for contact and photic stimuli aggregated in tentacles, palps, and eye spots. Whether receptors for chemical stimuli are similarly aggregated has not been determined.

Associated with the cephalization of the receptors, there is a cephalization of the ganglia of the central nervous system. In the earthworm the dorsal or cerebral ganglia (together with the subœsophageal ganglia, according to some authors) may be said to constitute the brain. This is in no sense the homologue of the vertebrate brain. In the marine worm, *Nereis*, there is a cephalization of a number of the ganglia, which in the earthworm would be considered as segmental ganglia, so that the so-called brain mass comes to be considerably enlarged.

Specialization of Nerves among Worms.—The nervous system of the lower segmented worms is located in the hypodermis, except at the most anterior region, where the cerebral ganglia are separated from the hypodermis by a definite boundary. In some of the lower forms the segmental character of the nervous system is hardly noticeable. No segmental arrangement of ganglion cells or of nerve fibers is to be found. The number of fibers given off from the ventral cord in any segment is a variable, as many as thirty-five having been counted in a single segment of *Axiothea torquata* or of *Clymene producta*. The nervous structures of these forms are very primitive and indicate a partially centralized nerve plexus.

In these forms there is a considerable specialization of nerve cells and fibers, there being developed, among others, groups of cells which are decidedly larger than the average, and to which the terms *giant cells* and *giant fibers* have been given.

Margaret Lewis studied particularly the giant cells and giant fibers of the two Polychætæous Annelids just mentioned. She found that the giant fibers are formed by the union of processes arising from the giant cells. In these forms there is no constancy in the number or arrangement of the giant fibers, though the number of the fibers is always limited. The cells from which they arise appear in the subœsophageal ganglion,

and are found scattered along the lateral and ventral portions of the nerve cord, without respect to metamerism or symmetry. A more extended discussion of the giant cells and giant fibers will be found in the accounts of the nervous systems of the earthworm and of the Crustacea.

The higher worms and leeches possess true central nervous systems, in the anatomical sense of the term. In each case the system is made up of a double chain of ganglia which extends nearly the whole length of the animal. The most anterior pair of ganglia lies dorsal to the pharynx, and is connected by means of fibrous commissures with the remainder of the chain (known as the ventral nerve chain). It is ordinarily stated that fibers pass from the ventral cord to the receptor organs and to the

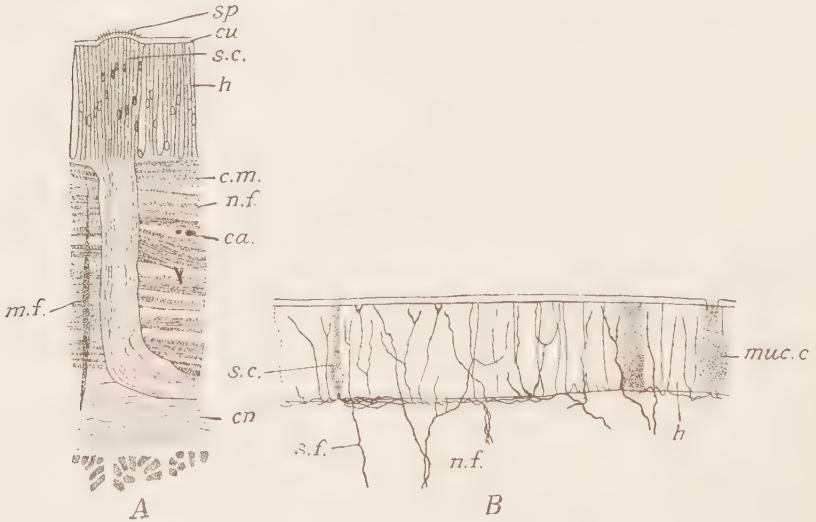


FIG. 114.—*Lumbricus*. *A*, sense bud. (*Ac. to Hesse, from Schneider.*) *B*, Epithelium impregnated with silver. (*Ac. to Retzius, from Schneider.*) *sp*, sensory processes; *cu*, cuticle; *s.c.*, sensory cells; *h*, hypodermis; *n.f.*, nerve fibers; *c.m.*, circular muscles; *m.f.*, muscle fiber; *c.n.*, circular nerve; *ca*, capillary; *muc.c.*, mucous cell.

muscles. Certain modifications of this statement, as regards conditions in the earthworm, appear in a later paragraph. It is reported that, among the leeches destruction of a portion of the ventral nerve cord leads to complete failure of nervous function in the segments affected. This certainly is not true in the earthworm. In the leeches the nervous system shows a different architectural plan from that found in the earthworm. In the leech, fibers run from the anterior end, while among the Oligochætæ there are no nerve fibers, so far as is known, which extend throughout the length of the body. Even the giant fibers are those of short neurones extending, according to Stough, at most for only the length of a single segment.

Receptor Organs of the Earthworm.—A number of investigators have furnished morphological evidence of the existence of receptors of different sorts in the earthworm. Figure 114 shows Hesse's drawing of a section of a sense bud and Retzius' drawing of finely distributed sensory nerve elements in the epidermis of the earthworm. In Fig. 115 a photograph of three sense cells of the epidermis of the earthworm, stained by methylen blue, is shown. The receptors shown in these figures may all be chemical in character. In addition to these, Hess has demonstrated the general nature of the photic receptors in the earthworm. It is possible that no

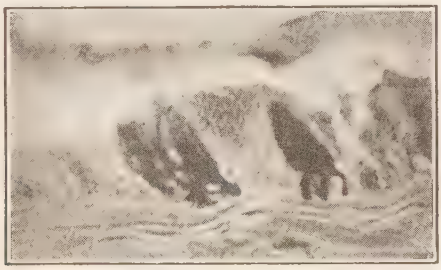


FIG. 115.—Sensory cells in epidermis of earthworm. These receptors are evidently different from those which have been designated as chemical receptors by Hesse and by Landgon. (*Preparation by W. N. Hess, photograph by author.*)

special receptor organs are developed in the earthworm for the sense of contact.

Physiological Dominance in Worms.—Among the worms there is not only an exhibition of the beginning of cephalization with the characteristic dominance of the body by the nervous elements of the anterior end, but there is also an exhibition of the dominance of each individual segment by the ganglia of the ventral nerve cord in each segment. Each segment is to be looked upon as a unit of structure. The coordination and the integration of these units into a harmonious worm body is brought about by the ganglia and connectives of the central nervous system, together with the subepidermal nerve plexus. The significance of this latter structure has apparently been overlooked by many investigators. It is not difficult to suppose that there is a correlation between the distribution of receptors in the body surface and the degree to which physiological dominance exists in the different regions of the body. Hess has made an estimate of the number of photoreceptors in certain measured areas (200×300 microns) of the surface of the body of the earthworm, with the following interesting result:

TABLE 53

Region of earthworm	Median dorsal area	Antero-lateral area	Latero-median area	Postero-lateral area	Ventro-median area	Anterior tip area	Total
Prostomium.....	18	..	12	..	5	22	57
Segment 1.....	10	0	12	0	4	..	26
Segment 2.....	5	0	5	0	0	..	10
Segment 3.....	3	0	4	0	0	..	7
Segment 40.....	0	0	1	0	0	..	1
Antepenult segment.....	1	0	1	0	0	..	2
Penult segment..	1	0	3	0	0	..	4
Last segment....	4	2	5	1	2	..	14
Totals.....	42	2	43	1	11	22	121

This investigation indicates that the prostomium of the worm is the most richly supplied portion of the body in so far as photoreceptors are concerned (being provided with about 700 in the nerve enlargements in the prostomium, and 400 in the epidermis of this portion of the body). It also indicates that the dorsal and lateromedian areas are about equally well supplied with these structures, as well as the fact that the middle region of the body is very poorly supplied. Whether all these receptors in different portions of the body are equally sensitive to light stimulation has not yet appeared. Certain it is that the number of receptors exposed to stimulation in the extreme anterior end of the worm is far in excess of the number elsewhere in the body surface. This means that there must be coming into the central nervous system, from the photoreceptors of the extreme anterior end of the worm, many times as many impulses as come into it from the receptors of the middle region of the body. If, as may readily be true, the receptors of the anterior end of the worm are more irritable than those of other regions, it would naturally follow that light falling upon this region would appear to have a greatly exaggerated stimulating effect as compared with similar illumination of other portions of the body.

The earthworm is provided with effective chemical receptors frequently arranged in groups. Whether these are of smell or taste, or both, is yet to be demonstrated. Nothing is known as to their number or distribution, though it seems safe to say that, in all probability, they are more numerous in the anterior end of the body than elsewhere.

Evidence of their effectiveness appears in the fact that the worms have power to exercise choice in the matter of taking in food substances. Experiments made during a number of years and involving the use of many hundreds of worms show that, when given a choice of several differ-

ent substances, they take meat and chocolate in preference to apple, turnip, or carrot, and that they take lemon and onion last of all.

Nerve Net and Nerve Plexus.—The meaning of the terms *nerve net* and *nerve plexus*, as they are here used, must now be made clear. The nerve net is an interlacing network of fibers, with cell bodies. The fibers branch and anastomose, and show definite protoplasmic continuity from one protoneurone to another. The nerve plexus is also an interlacing network. This term is often used synonymously with nerve net, and also is applied to net systems in which cell bodies do not appear, or in which they have not been demonstrated. The term nerve plexus is, therefore, the more general and more inclusive term. It probably is

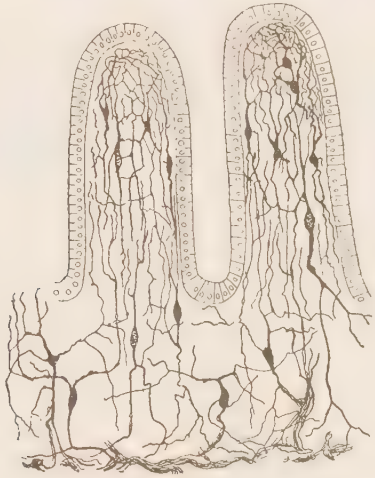


FIG. 116.—Plexus of sympathetic neurones found in the villi of the small intestine of the guinea pig. (*Redrawn from Herrick.*)

true that there are nerve plexuses in which synaptic connections between the different cells occur, though such arrangements are certainly not common among the lower forms. Figure 116 illustrates the form of nerve plexus found in the villus of the intestine of the guinea pig. Similar plexuses occur in many places.

The Nervous System of the Earthworm.—The nervous system of the earthworm is at the same time a retrospect and prophecy. It forms a sort of transition stage between the nerve-net condition of nervous organization and the more highly centralized systems of the higher animals. The system is really a double one, consisting of a central system and two nerve-plexus systems, the sensori-motor and the visceral systems (see Figs. 117 and 119). The sensori-motor system is itself a double nerve-plexus system, the outer portion (the subepidermal nerve plexus) lying underneath the epidermis and external to the basement membrane, and the inner portion (intermuscular nerve plexus) lying

within the musculature of the body wall. These networks are well developed, very closely interrelated, and composed of elements which are individually very small and exceedingly numerous. Whether the elements composing these networks are of the type of the protoneurones characteristic of the nerve net of the Cœlenterata is a matter still to be solved.

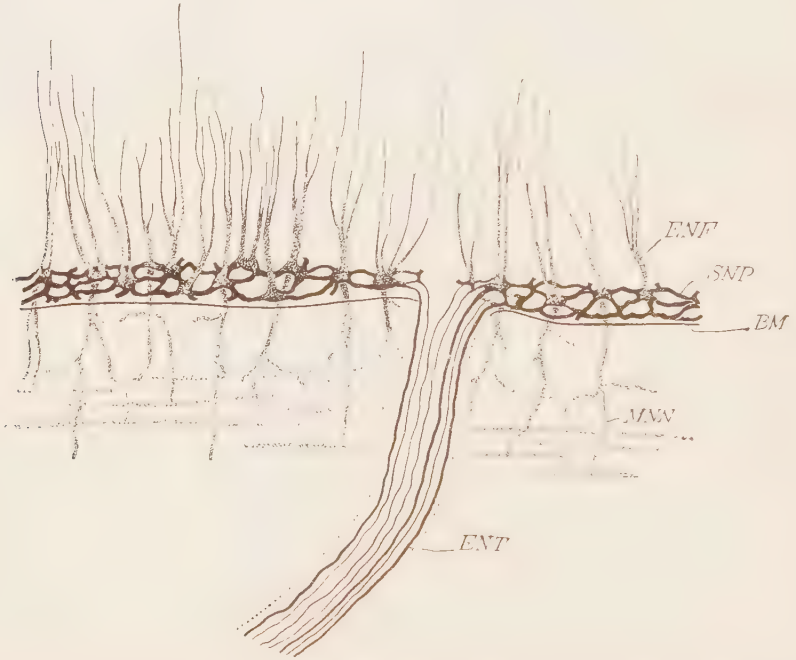


FIG. 117.—Drawing of the subepidermal nerve plexus from the anterior region of the prostomium of the earthworm, showing connections with the central nervous system, muscular nerve plexus, and epidermal nerve fibrillae, silver-nitrate technic: *BM*, basement membrane; *ENT*, epidermal nerve trunk; *ENF*, epidermal nerve fibrillae; *MNN*, muscular nerve plexus; *SNP*, subepidermal nerve plexus. (*This figure was drawn by Dr. W. N. Hess and is here reproduced by his kind permission.*)

The Subepidermal Nerve Plexus.—Then nervous system of the earthworm is of especial interest from the fact that some of the receptor cells connect directly with the subepidermal nerve plexus. From there, impulses may pass directly to the intermuscular portion of the plexus (Fig. 119), and so to the muscles of the body wall; or they may pass to the nerve cord. If they pass to the muscles, there is a semidirect stimulation of the muscle through the medium of a short reflex arc. If the impulses pass to the ventral nerve cord, there is a reflex stimulation of the muscle fibers; only, in this case, the response is slower because of the longer and more involved course along which the impulses must pass. The mediation of nerve impulses by the various adjustor cells of the ventral cord, and by cells of the cerebral ganglia may render the reflexes

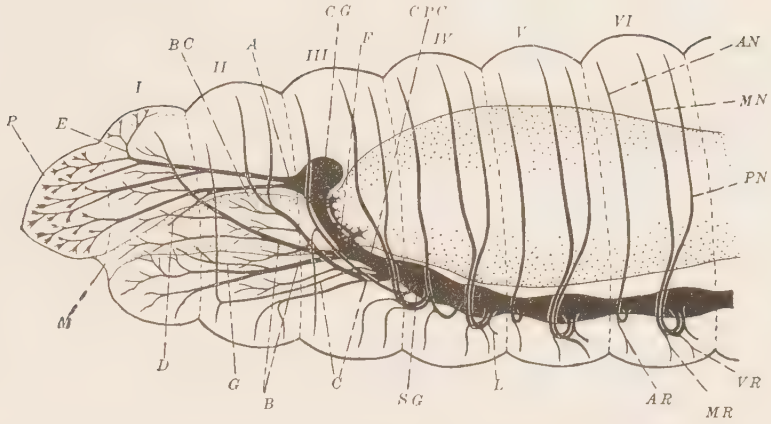


FIG. 118.—Drawing showing the arrangement of the larger nerve trunks in the left half of the anterior segments of the earthworm, *Lumbricus terrestris: A*, nerve from lateral region of cerebral ganglion which passes to prostomium; *AN*, dorsal ramus of anterior segmental nerve trunk; *B*, nerve from near middle region of circumpharyngeal connective which passes to segment one; *BC*, buccal cavity; *C*, paired nerves from the ventral region of the circumpharyngeal connective which pass to segment two; *CG*, cerebral ganglion; *CPC*, circumpharyngeal connective; *D*, branch of nerve to prostomium that supplies tissues of the dorsal region of the buccal cavity; *E*, nerve that supplies the portion of the prostomium in the dorsomedian region of segment one; *F*, pharyngeal plexus; *G*, branch of nerve to segment one, that supplies tissues of the ventral region of buccal cavity; *L*, visceral nerve; *M*, mouth opening; *MN*, dorsal ramus of median segmental nerve trunk; *P*, prostomium; *SG*, subpharyngeal ganglion; *VR*, ventral ramus of anterior segmental nerve; I to VI, segments one to six. (*The author is indebted to Prof. W. N. Hess for this figure.*)

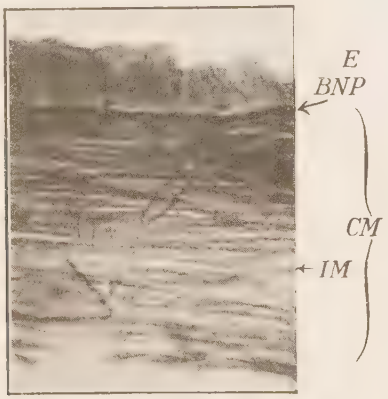


FIG. 119.—Section through the body wall of the earthworm, showing fibers from the sub-epidermal nerve-plexus passing downward through the layer of circular muscles toward the layer of longitudinal muscles which are not included in the photograph. *E*, Epidemis; *BNP*, Region of sub-epidermal nerve plexus and basement membrane; *CM*, Layer of circular muscles; *IM*, Inter-muscular nerve-plexus. The two large V-shaped darkbodies shown in the print are the images of dust particles. (*Photo by the author, from preparation by Smallwood.*)

very variable in character. The ventral nerve cord contains many motor cells, the fibers from which extend to the subepidermal nerve plexus. From this plexus, impulses are relayed to the intermuscular plexus and so to the muscle fibers.

The Central Nervous System.—The central nervous system of the earthworm consists of a pair of supraœsophageal ganglia (sometimes loosely called the brain) and a ventral chain of ganglia, a pair of ganglia lying in each segment of the body. These ganglia of the different segments are connected into linear chain by means of longitudinal connectives. Figure 118 shows a lateral view of the anterior end of the nervous system of the worm Lumbricus as described by Dr. Hess. The segmental arrangement of ganglia and the lateral nerves of the segments, shown in segments IV, V, VI, are repeated throughout the whole length of the worm.

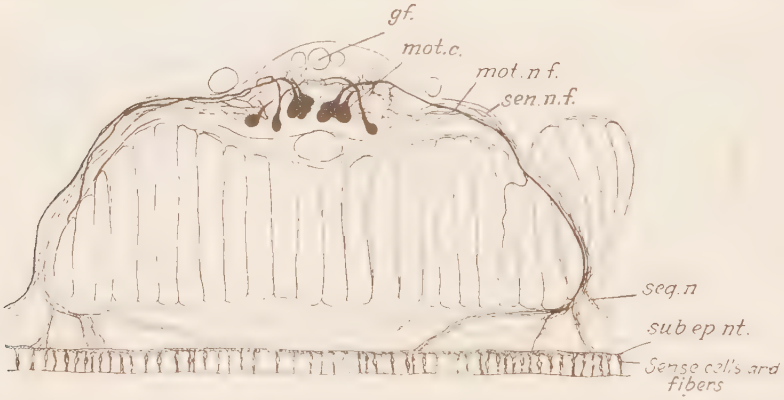


FIG. 120.—Cross-section of ventral nerve cord of *Lumbricus*, showing motor cells and fibers, and relation of ventral nerve cord to body wall—silver prepartion: *gf.*, giant fibers; *mot. c.*, motor cell; *mot. n. f.*, motor nerve fiber; *sen n. f.*, sensory nerve fiber; *seg. n.*, segmental nerve; *sub. ep. nt.*, subepidermal nerve plexus. (*After Retzius.*)

This external appearance of segmental is, however, relatively unimportant except in furnishing landmarks indicating internal, hidden, structural relationships.

Peripheral Nerves.—Each ganglion of the ventral nerve chain of the earthworm gives rise to three pairs of nerves (see Figs. 118 and 120). The first of these arises from the sides of the ventral nerve cord just behind the anterior septal wall: the second and third arise from the ganglionic mass itself. These nerve trunks pass laterally across the cœlom to the longitudinal layer and give rise to two branches or rami. The dorsal rami run between the circular and longitudinal muscles nearly to the middorsal line. The ventral rami run between the circular and longitudinal muscles nearly to the midventral line. The dorsal rami give off branches to the intersetal tract, and then many small branches which pass vertically or obliquely to the epidermis. Thus, three nerve rings—anterior, median, and posterior—are formed in each segment.

Dawson has directed attention to the fact that these nerve rings are not wholly fibrous in nature. Nerve cells appear along the courses of the rings. These cells are of four forms: tripolar cells, which are sometimes grouped in the intersetal tracts; spindle-shaped bipolar cells; crescent-shaped bipolar cells; long, slender, pyramidal cells. So far as the author is aware, the functions of these different cells have not been determined.

The Minute Structure of the Ventral Nerve Cord.—As the unit of structure of the nervous systems among the lower Cœlenterata has been

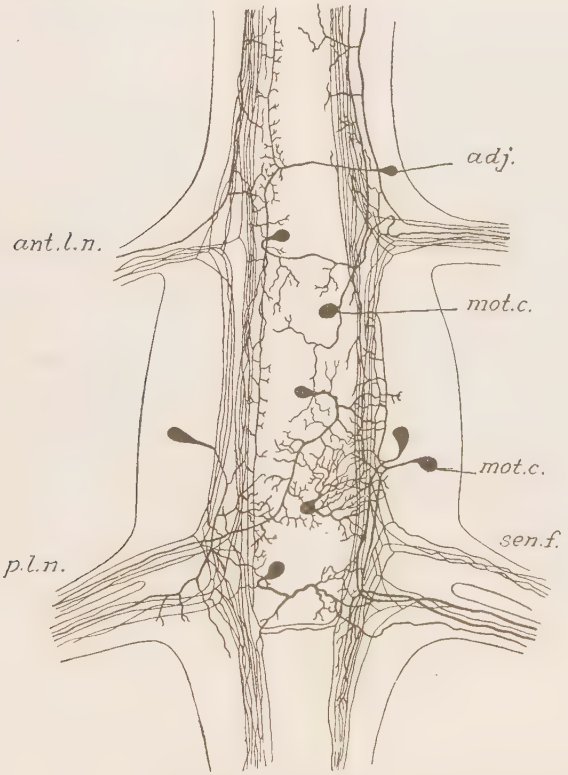


FIG. 121.—Ventral nerve ganglion of *Lumbricus*—Golgi method: *mot. c.*, motor cell; *adj.*, adjustor cell; *ant. l.n.*, anterior lateral nerve; *post. l.n.*, posterior lateral nerve; *sens. fib.*, sensory fibers. (*According to Retzius, from Schneider.*)

seen to be the protoneurone, here it is the neurone. Some idea of the relations existing between the different sets of neurones may be gained from an inspection of Figs 120 and 121. The different types of neurones, with their variously branching processes, will make possible an understanding of how a very slight and limited stimulation may result in a widespread reaction on the part of the worm. The neurones composing the central nervous system serve the general function of conduction in

three different ways. Some serve to transmit impulses from receptors in the surface epithelium of the body to the central nervous system. These are sensory in character and are spoken of as receptors. Some serve to transmit impulses from the sensory neurones to other neurones within the nerve cord. These are spoken of as internuncial, adjustor, or coordinating neurones. Some are given the function of transmitting impulses from the ventral nerve cord to muscle cells or other effector structures (Figs. 122 and 123). These are the motor cells.

A cross-section of the ventral nerve cord of the earthworm taken through a midbody region shows it to be oval in outline, and covered on

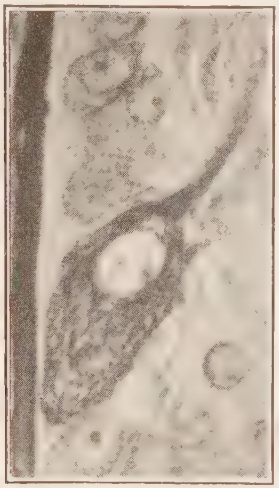


Fig. 122.

Fig. 123.

Fig. 122.—Photo of motor nerve cell of ventral nerve chain of the earthworm showing intracellular nerve net. (*Preparation by Dr. W. N. Hess, photograph by author.*)

Fig. 123.—Photograph of bipolar nerve cells located just at the point of origin of lateral nerve from ventral nerve cord of earthworm. One fiber penetrates the neuropile; the other passes out along the nerve trunk. (*Preparation by W. N. Hess, photograph by author.*)

the outside by a sheath made up of peritoneal epithelium, longitudinal muscle fibers, blood vessels, and a cuticle (see Fig. 124). Within the sheath are to be found the neuropile, the ganglion cells, and the giant fibers.

The *neuropile* is made up of a vast network of fine interlacing fibers which arise from nerve cells. Intermingled with the fine processes from the nerve cells are also strands of connective tissues and neuroglia cells serving as supports for the nervous structures. The neuropile is divided into two more or less distinct strands.

The *ganglion cells* are of two sorts, large and small. They are also of different classes, unipolar, bipolar, and multipolar. These ganglion cells send their processes into three bundles of large axones, dorsal, ventral

and lateral. The ventral bundle of axones is associated with, and frequently surrounds, the ventral giant fiber.

There are two groups of giant fibers in the ventral nerve chain. The dorsal group is made up of three giant fibers, a median fiber, and two lateral fibers. These lie above the neuropile. In the ventral part of the neuropile are the two ventral giant fibers. These are less conspicuous than the dorsal fibers.

The author is indebted to Dr. Stough for permission to use certain unpublished material in giving a description of these fibers. The median

FIG. 124.—Cross-section of nerve cord of earthworm: *M.S.*, muscle sheath; *G.C.*, giant cells, with fibers extending to commissure connecting lateral giant fibers; *Con.*, commissure connecting lateral giant fibers; *M.G.F.*, median giant fibers; *L.G.F.*, lateral giant fibers. (*By the kindness of Dr. Stough.*)

giant fiber extends from near the anterior surface of the suboesophageal ganglion, just posterior to the divergence of the circumoesophageal connectives through the whole length of the nerve cord, and terminates at the posterior end of the worm. This fiber increases in size as it passes posteriorly, and is largest in the middle and posterior regions of the body. In the extreme posterior segments of the body it decreases again in size. In each ganglion there are two pairs of giant cells which occupy a median position and are connected with the median giant fiber. There is also a pair of giant cells lying in a lateral position in each ganglion whose processes also enter this fiber (Fig. 124). The median giant fiber also receives processes from a number of the smaller ganglion cells. In some

cases there is a fusion of some of these processes before they enter the fiber.

The lateral giant fibers also extend throughout the length of the worm, except the extreme anterior and posterior ends. At the ends the fibers appear to taper out, so that it is difficult to determine just where they end. They reach their largest development in the midbody region of the worm. On the average, the sum of the cross-section areas of the two lateral fibers is equal to the area of the median fiber. In each ganglion there is a pair of giant cells connecting with the lateral giant fiber. At intervals, the fused processes of numerous smaller cells also enter the lateral fibers.

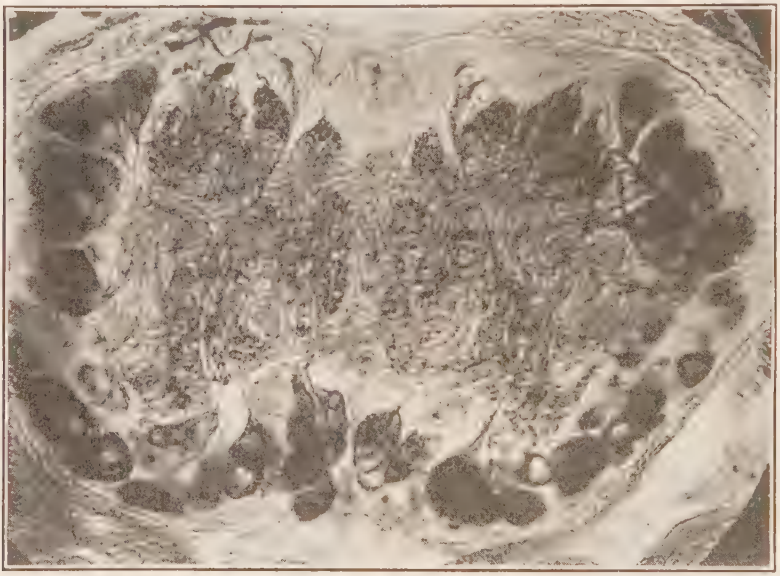


FIG. 125.—Cross-section of ventral nerve cord of earthworm, prepared by Cajal's method. Nerve cells about periphery, sending fibers into neuropile. Note also long fibers from center of lateral giant fibers extending well into neuropile. (*Photograph by author from preparation by Hess.*)

The lateral giant fibers are also connected with each other through the medium of a transverse bridge, according to Stough. The present author has failed to find evidence of these bridges in sections prepared by a technic different from that employed by Stough.

Stough contends that each giant fiber is made up of a large number of slender axones, so closely appressed as to make their outlines hexagonal, and each has a dark central point. In other words, Stough looks upon the giant fibers as large cables of fine nerve fibers. Other preparations give a somewhat different view of the matter. Figure 125 is a photograph of a section of the nerve cord of the earthworm showing large, dark, fiber masses reaching down from the central core of the giant fiber into

the masses of neuropile. This would seem to indicate that it is the central portion of the giant fiber which may be concerned with the work of conducting.

Each giant fiber is divided by segmental cross-partitions, the septa being continuations of the sheathes of the giant fibers normally closing off one segment of the fiber from the next. The septa appear to be diagonal sheets which are thrust across the fibers so that the fibers take on the appearance of overlapping wedges. The surface of contact between the adjacent fibers is thus very large.

Stough considers the giant fibers to be long, continuous structures reaching from end to end of the worm, and to be made up by the fusion of cell processes. Johnson was the first to show that the giant fibers of some of the Crustacea were segmented. In Astacus the lateral giant fibers are segmented, while the giant fibers of Palæmonetes are partially or completely fused (see Fig. 126). Except for the first few and last few segments, the same giant-fiber elements occur in each segment and in the same order in all the ganglia of the worm.

The general form and position of the fibers indicate a function of longitudinal transmission. Impulses must pass from one segment to another. To Stough, the septa seem to be synaptic in function, as they undoubtedly mediate the giant-fiber impulses as they travel through the cord. The size of the septa favors such a function, as there is there an increased area for synaptic transfer. Also, the septa are colloidal in nature. There is the further fact that, upon section, they do not degenerate beyond the limits of the cut segment. This leads to the suggestion that they are segmental structures. Stough says that, if the portion of giant fiber in a given segment were a single neuron, one would be justified in thinking of the septum as a synapse. Since many neurones are involved, he prefers the term *multiple synapse* with the understanding that the junction is between net-like combinations of neurones on one side and a similar structure on the other, and not merely between

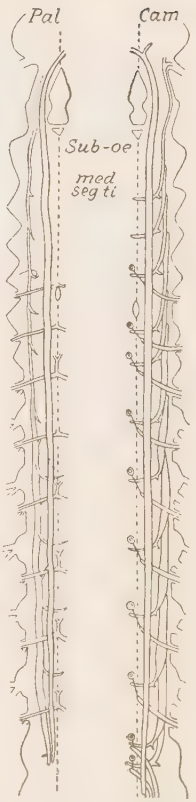


FIG. 126.—Diagram of the giant fibers of the right side of the nerve cord of *Cambarus limosus* and of the left side of the nerve cord of *Palæmonetes vulgaris*. The broken line running lengthwise through each drawing indicates the median sagittal plane of the body. The connectives between the ganglia are very much shortened except in the thorax of *Palæmonetes*, where they are normally very short: *Sub-oe*, region of suboesophageal ganglion; *med*, median giant fiber, which in each form runs a continuous course; *seg ti*, segmental giant fiber whose posterior termination is in the first thoracic segment. Note that this arrangement is repeated in succeeding segments.

neurones. The giant fiber system is also probably polarized, and it seems likely that the median giant fiber conducts in one direction and the lateral giant fibers in the opposite direction.

The term *nerve net* should be applied to the giant fiber system only with caution. The nerve net is usually conceived to be a fusion of neurones with diffuse conduction and without polarization. This condition does not exist in the earthworm. The presence of junctions which are functionally synaptic also limits the applicability of the term in a consideration of the giant-fiber system. In another sense the term *nerve net* may be applied; that which recognizes in the giant fibers a polarized system of neurones which are associated segmentally in net-like combinations, each of these nets coming into synaptic relations with similar nets in contiguous segments of the cord.

The Minute Structure of a Ventral Ganglion.—The finer structural details of the central nervous system of the earthworm have engaged the efforts of a large number of investigators, among whom may be named Friedlander, Haller, Cerfontaine, Retzius, von Lenhossek, and others. It would hardly be expected that these investigators would be in entire agreement as to the types of neurones involved in the building up of such a nervous system as is found in the earthworm, or as to the manner in which these neurones are arranged.

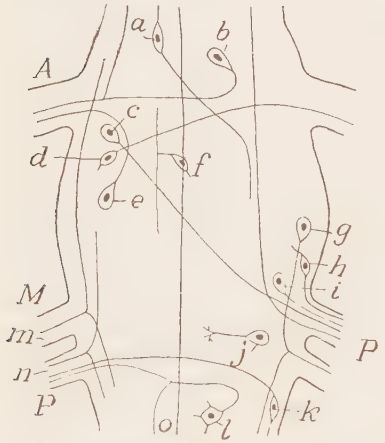


FIG. 127.—Diagram illustrating cell structure of the ventral chain ganglia of *Lumbricus: A*, anterior segmental nerve; *M*, median segmental nerve; *P*, posterior segmental nerve; *a* to *l*, ganglion cells; *m* to *p*, nerve fibers. (*After Cerfontaine.*)

The first general statement that may be made is to the effect that all sensory cells lie outside the central nervous system, and, in general, at the periphery of the body. They may occur as single, isolated receptor cells, or they may be grouped into small masses as indicated in Fig. 114, and as has been demonstrated by Langdon. The cells which are found in the central nervous system are, then, motor cells and internuncial or associational cells. These cells tend, on the whole, to be arranged in a bilaterally symmetrical manner.

The following account is a very much condensed statement as to the types of cells and their location in the ventral nerve cord of the earthworm as determined by Cerfontaine, and reviewed by Fortuyn. It will be remembered that three pairs of nerves arise in each segment of the worm. These may be called *anterior segmental nerve*, *median segmental nerve*, and *posterior segmental nerve*. Cerfontaine found the following cell groups (see Fig. 127):

1. Cells whose fibers run into the anterior segmental nerve. Of these there are three groups:

a. Unipolar ganglion cells, whose bodies lie ventrally and near the midline of the cord, and whose processes leave the ventral nerve cord dorsally in the anterior segmental nerve of the opposite side. Just before the nerve leaves the cord, a lateral process is given off which passes anteriorly in the connective toward the next preceding segment.

b. Bipolar cells, whose bodies lie in the lateral portion of the cord. Their long processes cross through the neuropile to the opposite side and leave the cord in the anterior segmental nerve. The dendritic processes of these cells are very short.

c. Unipolar cells, laterally placed in the cord. Their processes pass forward and leave the cord in the anterior segmental nerve of the same side.

2. Cells whose fibers run in the median segmental nerve. Of these there are three groups:

a. Unipolar cells whose bodies lie near the level of origin of the anterior segmental nerve, and whose processes pass obliquely through the ganglion to the median segmental nerve of the opposite side.

b. Unipolar cells which lie near the base of the median segmental nerve, and whose processes leave the cord by the median segmental nerve of the same side.

c. Bipolar cells with short dendrites, whose long neurites leave the cord through the median segmental nerve of the same side.

3. Cells whose fibers leave the cord through the posterior segmental nerve. Of these there are three types:

a. Unipolar cells lying in the mediolateral portion of the ganglia The axis cylinders arising from these cells divide into two as they reach the base of the posterior segmental nerve, one branch passing out along the nerve, and the other passing posteriorly into the connective leading to the next posterior segmental ganglion.

b. Bipolar cells, lying posteriorly to the origin of the posterior segmental nerve. One process passes posteriorly into the connective; the other crosses the median line and passes out of the cord along the posterior segmental nerve of the opposite side.

c. Multipolar cells. These lie in the cord posterior to the origin of the posterior segmental nerve. Their axis-cylinder processes cross the median line, and divide dichotomously. One branch passes posteriorly in the other side of the cord, and the other branch passes out through the posterior segmental nerve of the opposite side.

In addition to the cells mentioned above there are the following:

a. Bipolar cells, located near the origin of the anterior segmental nerve, which send one process anteriorly in the connective of the same

side, and the other process backward obliquely into the neuropile of the opposite side.

b. Bipolar cells located near the midline. Each gives off a fiber which divides, one branch going anteriorly and the other posteriorly in the same side of the cord.

c. Cells at the level of median segmental nerve, which send their processes into neuropile of the same side.

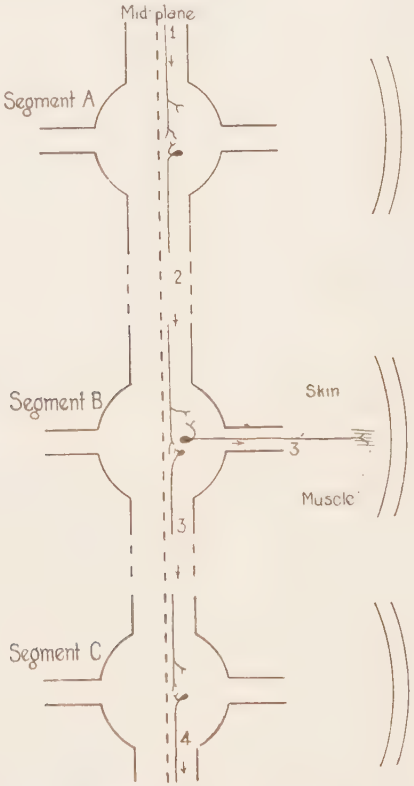


FIG. 128.—Diagram of the chains of nervous elements within the central nervous system of the earthworm, which are concerned in the longitudinal conduction of nervous impulses. The nerve cells successively activated are numbered 1 to 4. At each segmental ganglion these with peripheral nerve cells, one of which is indicated by 3′ (see the text). (*From Herrick, by permission of Henry Holt and Company.*)

There are also in the cord three fiber bundles made up of the processes of sensory cells which make their way into the cord along the three pairs of segmental nerves.

A very much simplified scheme of the types of nervous transmission found in the earthworm is shown in Figs. 128 and 129. Simple longitudinal conduction is considered by Herrick to involve the paths shown in Fig. 127. This is definitely conduction through a chain of neurones,

the impulses passing on from one neurone to another through synaptic arrangements in each segmental ganglion. There is also provided, in each ganglion, a synaptic connection with a peripheral motor nerve element.

Everyone who has observed with any care the locomotor movements of the earthworm will agree with Herrick that they involve a more complex series of nerve elements than is indicated in Fig. 128. Herrick has shown in his "Neurological Foundations of Animal Behavior" a figure (repro-

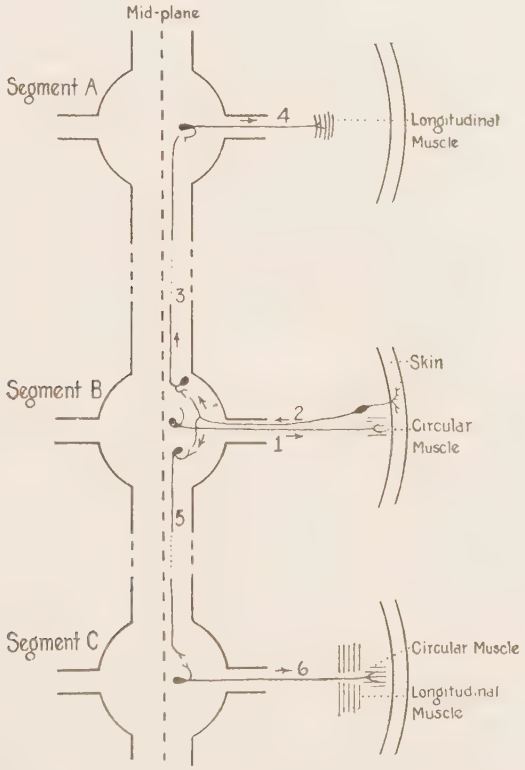


FIG. 129.—Diagram of the nervous elements concerned in ordinary locomotor movements of the earthworm in three segments of the midbody region in successive phases of the locomotor process. (For description see text.) (*From Herrick, by permission of Henry Holt and Company.*)

duced here as Fig. 129) which shows the possible arrangement of neurones involved in only one out of the whole series of neuromuscular reactions. Three segments in the midregion of the body are chosen, and designated *A*, *B*, *C*. These are in successive phases of locomotor movement. *A* is taken when it is in its shortening process, *B* is in its elongating phase, *C* is in its shortening phase. Each of these segments is to be regarded as representative of the whole group of segments in like phase. The segments *A* and *B* and between *B* and *C* are omitted.

In his discussion of this matter Herrick says:

Assuming that segment *B* is in the phase of contraction of the circular muscles which have been excited by motor neuron (1) with the longitudinal series of muscles relaxed, the resulting elongation of the body in this region exerts traction in the skin and underlying tissues which serves as a stimulus for a sensory neuron (2) which passes inward from the skin to end in the ganglion. Here, the nervous impulse is passed on to two other neurones, one of which (3) transmits it upward, and the other (5) downward. The ascending impulse is taken over in segment *A* by a peripheral motor neuron (4) with resulting contraction of the longitudinal muscles of this segment and shortening of the body in this region. The descending nervous impulse is taken over in segment *C* by a peripheral motor neuron (6) which excites contraction of the circular muscles of this segment, thus propagating downward the phase of body elongation now in progress in segment *B*. In this way the successive phases of the locomotor movements pass downward along the length of the body.

This account, however, includes only a part of the process. As each of the muscles is excited to contraction, there is probably relaxation of its antagonist (reciprocal innervation). Moreover, the nerves and muscles of both sides are acting synchronously. The nervous apparatus involved in these aspects of the total movement do not differ in principle from those here pictured, and it will be unnecessary to consider them in detail.

Functions of the Neuropile.—No suggestion has been made as to the significance of the intricacy of the feltwork of nerve fibrils which make up the neuropile occupying the central portion of the ventral nerve cord. The mass appears to be made up of an almost hopelessly entangled mass of processes of nerve cells, the fibers running in all directions, and being the finely divided terminal processes of sensory cells, of internuncial cells, and the dendrites of motor cells. Through these processes, synaptic communication from cell to cell may take place. It will be evident that impulses coming in from a single afferent neurone may become widespread, and that impulses from a number of afferent neurones may be concentrated upon a lesser number of motor elements.

Operation of Nervous System of Earthworm.—The rather simplified account which has been given of the structure of the ganglia of the ventral nerve cord may have conveyed the impression that the central nervous system of even so lowly a form as an earthworm is a rather complicated mechanism. No account has been given of the structure of the cerebral ganglion, and none is to be given. These ganglia are somewhat more complicated structures than the ventral ganglia. They receive very numerous afferent nerve fibers from the receptors of the anterior end, and they serve to mediate the impulses coming to the central nervous system through these avenues. They also receive impulses coming up from the ventral nerve cord, and serve to mediate these as well. The main idea which it is desired should be established in this discussion is the fact that even in so simple an animal as an earthworm

there are many possible pathways over which impulses may travel, many possible reflex arcs, and, as well, many possible forms of response which may follow a given form of stimulation, or the stimulation of a given region of the body.

It has already been pointed out that the greater number of receptors at the anterior end of the animal must mean a greater shower of afferent impulses entering the central nervous system at this point. It is also very likely true that these receptors have a lower threshold of stimulation than receptors of the same type in other portions of the animal body. Weaker stimuli will then be effective in bringing about reactions. It is probable also that the motor nerve cells of the cerebral ganglia have a higher degree of specialization and a somewhat more delicate balance than do the cells of other parts of the central nervous system. The cells of the cerebral ganglia are, therefore, in position to respond to stimulation, from whatever source, more readily than are cells of the ventral ganglia. The higher degree of irritability, coupled with the greater flow of afferent impulses, serves to place the cerebral ganglia in a position of dominance in so far as the control of body movements is concerned. This dominance is maintained in part by the fact that the cerebral ganglion cells tend to occupy the outgoing paths with positive motor impulses, and in part by the inhibition of reflexes which would otherwise take place as the result of stimulation of the body surface at any random points.

It will be recalled that Loeb concluded that in the worm Thysanozoön, spontaneous movements were a function of the part of the body which contained the brain. He offered the suggestion that the failure of the decapitated worm to make spontaneous progressive movements may be the result of physical or chemical conditions, and that some alteration in the environing conditions may lead to spontaneous movements. A. R. Moore has experimented with the Polyclad worm *Yungia aurantica*. This worm behaves like Thysanozoön in that decapitated worms do not show progressive movements. Moore has succeeded in showing that decapitated worms still possess in the segmental ganglia the machinery necessary for progressive movement. This was accomplished by the use of strychnine and nicotine. Moore has also succeeded in bringing about progressive movements in decapitated worms by proper methods of stimulation. This may be accomplished by mechanical stimulation of the anterior median locus of the decapitated pieces. Chemical stimulation with a solution of phenol, 1 to 25,000 in sea water, brings about progressive movements which are like normal movements except that they are somewhat slower. He also found that a great alteration of the ratio of sodium and potassium ions to the calcium and magnesium ions was sufficient to bring about movements, though they were not of a sort which could be called progressive.

From an anthropomorphic point of view, it would be natural to suppose that the very numerous connections of the cerebral ganglia with various types of sense endings in the anterior end of the earthworm —and in other worms also—must be indicative of some peculiar directive influence which these ganglia play in the life of the animal. The cerebral ganglia of the earthworm do not have immediate control over normal progressive movements. They receive fibers coming from an exceedingly rich supply of very sensitive receptor cells, and so are in position to respond to impulses which would make little or no impression on other portions of the body. They may relay these impulses to the ventral nerve cord and so initiate locomotor movements. They may also inhibit movements which would naturally follow wholesale stimulation of the ventral nerve cord, as when the anterior end of the worm is cut off the worm writhes. The ganglia in direct control of coordinated locomotor movements are those of the ventral ganglionic chain. These may be set into action by stimuli arising in the same segment in which they lie, or which are conducted to the segment by fibers from the preceding or succeeding segment, or by impulses which may arise in the cerebral ganglia as the result of the stimulation of some of the many receptors intimately associated with them. While the anterior end of the worm is very sensitive to light, it is just as true that other portions of the worm are also equipped with receptors for light. Worms which have been deprived of their cerebral ganglia are still able to respond to differences in light intensity and color. These worms are positively heliotropic in behavior, while uninjured worms are negative to ordinary intensities of light. They are also sensitive to contact, as is shown by their stereotropic response when they come into contact with solid objects, and sensitive to certain chemical substances, as is shown by the fact that they tend to seek and bury themselves in compost, even though the cerebral ganglia are destroyed. Worms which have been deprived of the cerebral ganglia differ from the normal worms in that they tend to crawl in either direction. Their reactions are therefore determined by the impulses which come to them from the general body surface, and which are mediated through the segmental ganglia of the ventral chain.

Even in the absence of the ventral nerve cord, it is possible for the worm to execute movements which appear to be coordinated; but the coordination extends through only a few segments. Such movements are undoubtedly brought about through the mediation of the subepidermal nerve plexus. This structure has been left entirely out of consideration by most of the investigators who have attempted to account for the behavior of the worm under fixed and controlled conditions.

Some of the more important results which have been obtained in studies of the reactions of the earthworm, Lumbricus, and the marine worm, Nereis, are summarized in the following statement:

Condition	Earthworm	Marine worm
Normal	Shows movements which can be interpreted as purposive.	When placed upon sand will burrow into it.
	Seeks and burrows into compost.	In an aquarium will crawl along the sides of the glass.
Brain present	Negatively phototropic. Tends to inhibit reflexes.	Tends to inhibit reflexes.
Decapitated	No purposive progressive movements.	Crawls about on sand unceasingly.
	Crawls in either direction.	Does not burrow into sand unless anterior end is covered, when it burrows rapidly and secretes mucous to form a tube.
	Reaction time same as that of normal worm.	Does not withdraw from obstacles but attempts to go through them.
	Have same reactions as normal worm as regards crawling upon or into compost.	In the aquarium, attempts to go through the glass at corners.
	Positively phototropic.	When cerebral ganglion is removed connections between the head and the rest of the body are broken.
		Stimuli which affect receptors of anterior end no longer produce backward movements.
Loss of subœsophageal ganglion.		Leads to quiet. No attempt to burrow. Motor nerves for burrowing arise from this ganglion.
		Animal cannot eat. Loss of head-organ, the mouth, responsible for failure to burrow.
Segmental ganglia	In immediate control of locomotion.	Directly control muscular movements involved in burrowing and swimming.
	Control tropic responses to contact, light, chemicals.	
Nerve net	Serves to mediate impulses between receptors and ventral cord, receptors and muscles, ventral cord and muscles, and, probably, between immediately adjacent segments.	
Giant fibers	Provide for very rapid conduction of impulses through whole length of worm.	

Bovard, following the work of Friedlander (1894), has made an investigation of the transmission of the nervous impulse in the earthworm. Friedlander had stated that the central nervous system plays only a minor part in the normal creeping movements of the earthworm. He found that it was possible to cut worms into two pieces, and by joining these with a thread to provide for perfectly coordinated movements. It was also possible to remove from the body of the worm a piece of the nerve cord extending through ten or twelve segments and still provide for perfect coordination of movement.

Progressive movements consist of, first, a contraction of the circular muscles of a few segments at the anterior end. The setæ become directed backward and take hold upon the substrate, while the longitudinal muscles begin a contraction which pulls the next few segments forward.

The circular muscles of each segment contract, one after another, beginning at the anterior end and proceeding posteriorly. Immediately following the contraction of the circular muscle fibers, the longitudinal muscles contract so that a wave of extension followed by a wave of shortening can be seen slowly to traverse the whole animal. This is a beautiful illustration of the alternate activity of opposing muscles.

Reciprocal Innervation in Earthworm.—Garrey and Moore have found that this alternate activity of opposing muscles is really a case of reciprocal innervation, and, therefore, in line with Sherrington's reciprocal inhibition of antagonistic muscles as found in the vertebrates. Knowlton and Moore have carried the investigation a step farther, and have shown that, when the worm is under the influence of strychnine, there is a breakdown of synaptic resistance, such as would occur among the vertebrates, and as a result there is a contraction of both anterior and posterior parts of the worm when the part between them is stimulated.

Friedlander's idea was that the nervous impulses involved in locomotion were simple reflexes, involving only one segment at a time, and that the individual segment received its stimulus from the muscular pull of the next anterior segment. A more plausible explanation, in view of present information, is that the impulses involved are transmitted through the subepidermal nerve plexus, a structure of which Friedlander knew nothing.

Bovard, in his work, made use of a method devised by Biedermann, in which the middle portion of a worm was anesthetized by alcohol and nitric acid. The anterior and posterior ends would be left active, and the nerve cord of the anesthetized portion would still be able to transmit impulses. Bovard found as the result of an extended investigation that the spontaneous movements of the earthworm are due to the activity of the central nervous system. The muscular tissue of the worm is not rhythmically contractile. The experiments showed that the nervous impulse may be conducted in both directions through an anesthetized region of worm. Therefore, both ascending and descending neurones are present in the cord. The muscular tension or pull which seemed to Friedlander of so great importance in normal progressive movement could be eliminated, and the locomotor stimulus would still pass on down the cord for some distance, and when the tension was so eliminated by pinning the worm to a cork on a glass plate the posterior part of the worm could be observed to begin rhythmic movements of contraction which were coordinated with those of the anterior part. If the anesthetized portion was only a few segments in length, the coordination was almost perfect, and the beginning of the movement of the posterior part would follow in a shorter time than when the anesthetized portion occupied many segments. Stimulation through twenty segments was

effective, through twenty-eight segments rarely so, and through thirty segments was never effective.

Bovard also made preparations in which he dissected the nerve cord free, so that the anterior surface of the worm was connected with the posterior only by a nerve bridge. In such a preparation the locomotor impulses could be transmitted through considerable distances—more than ten segments easily, over twenty segments in favorable cases, and over twenty-eight segments rarely. There was no record of the transmission over any larger number of segments. The longer the bridge the greater the difficulty found in transmitting the nerve impulse. Normal stimuli in normal worms do not pass the whole length of the cord. They die out in transit. There are sometimes summation effects of weak impulses which pass through a considerable length of cord and then give rise to a contraction.

The rate of transmission of the nerve impulse is very variable. It may be as fast as 100 millimeters per second, or it may be so slow that it will die out in the cord, never emerging from an anesthetized region. The average rate of transmission is about 25 millimeters per second. One is tempted to raise the question at this point of whether Bovard's use of the term "slow" is fully justified. Was not Bovard dealing with a region of decrement in intensity rather than with a slowing of rate?

End-to-end movements are probably accomplished by impulses carried by the giant fibers. These fibers run the whole length of the animal and carry impulses at the rate of 1500 millimeters per second.

A study of the anatomy of the nerve cord shows that it possesses no long fibers. The processes of a given cell may join successive ganglia, but none extend for any great distance through the cord except the giant fibers.

The peculiarities of the locomotor impulses in transmission, such as the variability in the rate of speed, and the slowness of it, can be accounted for on the basis of the structure. The impulse, to make its way down the cord, must pass in each ganglion at least one synapse, and the possibility is that there would be more than this. Each synapse would not only cut down the strength of the impulse but would also slow down the speed because of the time consumed in crossing the gap between neurones. In normal creeping, the impulses travel regularly down the cord because each contraction of circular and longitudinal muscle in each segment sends in locomotor impulses which reinforce the impulse passing down the central nerve cord, and any loss through the synapse is made up in this way. If for any reason the muscular activity fail or if the nervous connections to the cord be destroyed the locomotor impulse traveling down the cord in this region would decrease in strength and in rate because of the lack of reinforcement.

TABLE 54.—SUMMARY OF RATES OF NERVOUS TRANSMISSION IN WORMS (*Carlson and Jenkins, from Bovard*)

Species	Direction	Centimeters per second
Cerebratulus	$P \rightarrow A$	5.4–9.0
Aulastoma lacustre	$P \rightarrow A$	56.0
Cirratulus sp	$P \rightarrow A$	90.0
Arenicola sp	$A \rightarrow P$	120.0
Bispira polpmorpha	$P \rightarrow A$	694.0
Aphrodite sp	$A \rightarrow P$	54.0
Polynoe pulchra	$P \rightarrow A$	293.0
Sthenelais fusca	$P \rightarrow A$	205.0
Eunice sp	$P \rightarrow A$	466.0
Nereis sp	$P \rightarrow A$	165.0
Nereis virens	$P \rightarrow A$	89.0
Nereis virens	$A \rightarrow P$	73.0
Lumbriconereis sp	$P \rightarrow A$	45–241.0
Lumbriconereis sp	$P \rightarrow A$	49–937.0
Lumbriconereis sp	$A \rightarrow P$	42–160.0
Glycera rugosa	$A \rightarrow P$	433.0
Glycera rugosa	$P \rightarrow A$	435.0

Muscle Tension and Reflexes in the Earthworm and Nereis.—A. R. Moore made use of preparations of the earthworm in which the cutaneous receptors have been anesthetized by placing the animals in M 8 magnesium chloride solution, which was allowed to act until stroking with a moistened camel's-hair brush would elicit no response. Moore found that when a piece was cut from the median region of the worm and suspended so as to make kymograph records, no spontaneous contractions would occur unless the muscle was weighted. If the muscle was weighted with a load of 1 to 2 grams, there was an immediate extension of the muscle strip followed by a series of contractions of the longitudinal muscles as long as the weight continued to act upon the muscle, or until the muscle became fatigued. It is to be noted in this experiment that, when tension is the exciting cause, the first phase of the reaction is an extension. This involves a contraction of the circular muscle fibers. If dermal stimulation is the exciting cause, extension is the first phase only if the stimulus is applied at the anterior end of the preparation. "It is evident that receptors for tension exist in the musculature of the worm." Since stretching of the longitudinal muscles brings about contraction of the circular muscles, it is clear that the receptors which are stimulated by tension must be connected with the circular muscles. Dawson has figured nerve cells lying in the ventral musculature, which he interprets as sensory cells. The existence of the subepidermal nerve plexus also makes the necessary structural provision for such a reaction as has been indicated.

The Homostrophic Reflex.—Morgulis has shown that, when the posterior segments of an earthworm are deflected in a given direction, the worm turns its anterior end so that the axis of the anterior segments tends to lie in a line parallel to the axis of the posterior segments. Moore interprets this action as the result of reflexes arising from unequal tension of the musculature of the body wall. He has shown that the receptors involved lie within the muscle wall, and that the conducting pathways lie within the ventral nerve cord, since section of the ventral nerve chain at a point anterior to the point of flexion prevents the completion of the reflex. Moore terms this reaction, which results in the course of progress being brought into line with the enforced orientation of the posterior part of the worm, the *homostrophic reflex.* Moore finds, also, that the receptor elements involved in this reflex are distributed through practically the whole length of the worm, while the effector elements are limited to the anterior twenty segments.

Among more highly differentiated forms, this homostrophic reflex plays a relatively less important rôle than in the earthworm, since in the higher forms there are developed receptor organs of considerable variety and efficiency. Even in the rather closely related form Nereis, there is to be noted some difference in the quality of the reflex. In Nereis the homostrophic response takes place as it does in the earthworm; the receptor elements are distributed through nearly the whole length of the body; the ventral nerve chain serves as the conducting pathway; the section of the nerve cord at any point posterior to the subœsophageal ganglion abolishes the reflex. Here, the effector elements are much more highly concentrated in the anterior end of the nerve cord than in the earthworm. Removal of the head of the worm greatly weakens the speed and definiteness of the reaction. The mediation of the reflex by the brain and the anterior two or three segmental ganglia is consistent with the high degree of cephalization found in this animal.

Homostrophic Reflex in Diplopods.—Among the Arthropods there is difficulty in finding forms which are sufficiently flexible to show the homostrophic reflex. The Diplopods *Julus venustus, Parajulus penn.*, and Polydesmus furnish suitable material. Crozier and Moore found, in a study of the forms mentioned, that a unilateral tension of the body affects the orientation of the head, and also the direction of locomotion, in a way similar to that in which it affects the Annelids. In these experiments it was found desirable to work under red light and to remove the antennæ so as to reduce the variety and intensity of stimuli coming to the central nervous system through the sensory organs of the head. When the animal is placed upon the table in a curved position, it tends at once to place the long axis of the head in a line parallel to the axis of the tail. If the animal is creeping actively and the tail is bent gently to one side, the axis of the head is immediately brought into a line parallel with the

tail axis. In order to bring about these effects, tension must be applied between four or five segments from the anterior end and the fourth from the posterior end. The terminal segments cannot be bent. Receptors for this reaction are probably distributed through the body as in the Annelids. Cutting of the ventral nerve cord at any level destroys the reaction. The removal of the head leaves the animal capable of living for several days, capable of locomotion, but with no trace of the homostrophic reflex. In this case the brain seems to be an essential part for the motor part of the reflex.

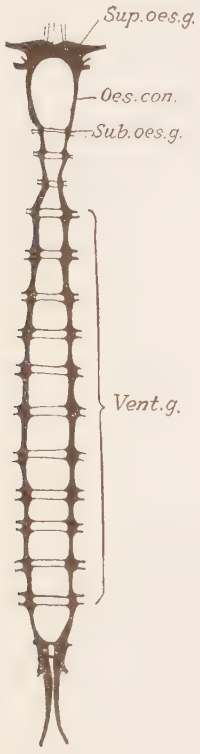


FIG. 130.—The central nervous system of *Branchipus venalis: Sup.oes.g.*, supra œsophageal ganglion; *oes.con.*, œsophageal connective; *sup.oes.g.*, subœsophageal ganglion; *vent.g.*, ventral ganglionic chain. (*After Hilton.*)

Nervous Organization among Arthropoda.—Numerous investigators have studied in some detail the nervous systems of many of the Arthropoda. Retzius, Bethe, Kenyon, Haller, Binet, Berlese, Hilton have all given accounts of the structure of one form or another. These accounts help to give us an understanding of the ways in which these different systems operate. It is not the purpose here to enter into any exhaustive discussion of the structures involved, but simply to point out in a very general way the plan of organization of the nervous system in two of these higher invertebrates. The forms presented are the Phyllopod Branchipus and the aquatic insect larva *Corydalis cornutus*.

It is among the Crustacea and Insecta that the ladder type of nervous system attains its most diagrammatic, and also its greatest, development. The form and the general arrangement of the nervous system of the Phyllopod crustacean Branchipus have been described by Hilton. A short review of the structures found in this form will assist materially in making clear the structure and mode of operation of the nervous systems of other forms, higher in the animal scale, for all are apparently constructed upon the same fundamental plan. This fundamental plan is, in many forms, very well concealed or obscured by the disproportionate development of certain parts of the system. The centralization and the cephalization of ganglia, which occur in many of the higher Arthropoda, tend to obscure the typical form of arrangement.

In Branchipus the nervous system (Fig. 130) is very clearly of the ladder type of structure, which has been described as being characteristic of other lower Crustacean forms. It consists of a supraœsophageal ganglion, with well-developed optic nerves arising from its frontolateral aspects, and two other pairs of nerves going to the antennæ and anten-

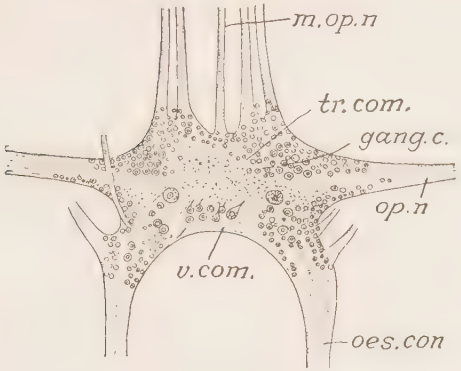


FIG. 131.—Brain of *Branchipus* from a surface preparation. The cephalic side is to the top: *m.op.n.*, median optic nerve; *tr.com.*, transverse commissure; *op.n.*, optic nerve; *v.com.*, ventral commissure; *oes.con.*, œsophageal connective; *gang.c.*, ganglion cells. (*After Hilton.*)

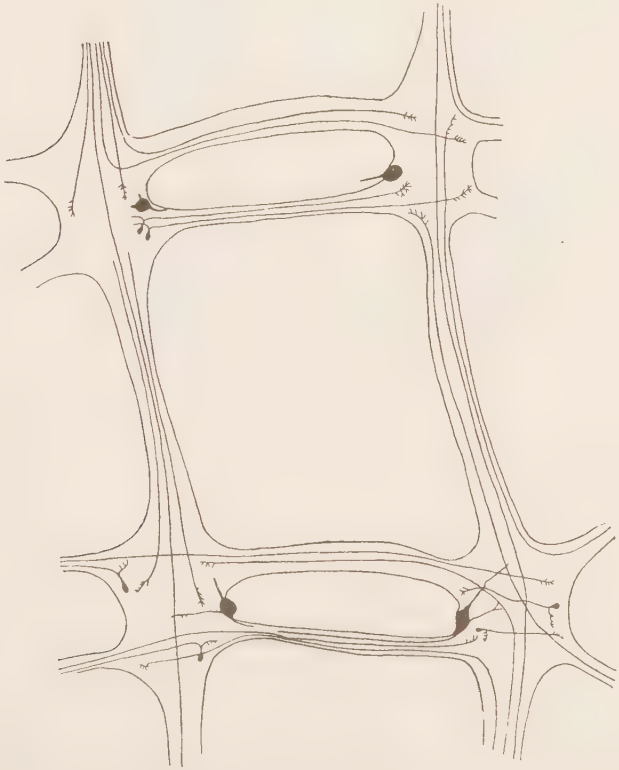


FIG. 132.—Diagrammatic plan of cell arrangement in the ventral ganglia of *Branchipus*. (*After Hilton.*)

nules arising more posteriorly, on or near the œsophageal connectives. Anteriorly, there are given off from the supraœsophageal ganglion, or *brain*, a median nerve going to the median eye, and two pairs of small nerves lateral to it.

From the outer borders of the supraœsophageal ganglia, there pass backward two long, nearly parallel strands. In each segment of the body below the brain there is ordinarily a ganglionic enlargement containing numerous nerve cells on each of these strands. Each ganglionin enlarge-

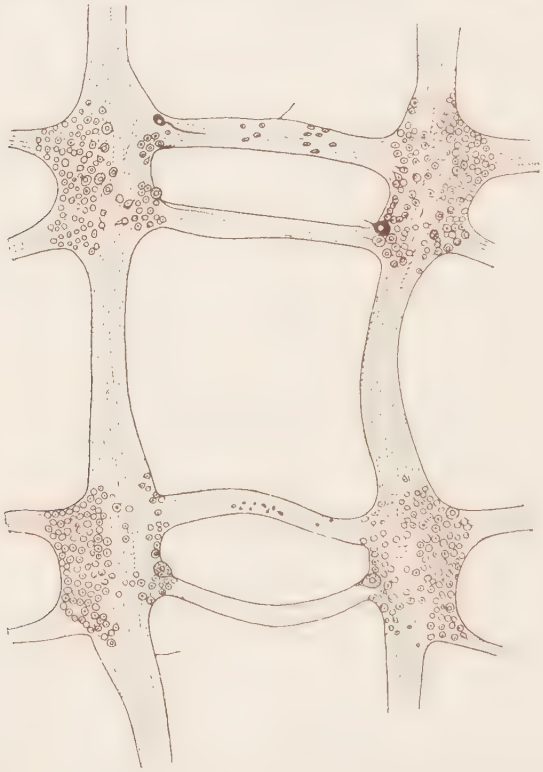


FIG. 133.—Sixth and first ventral ganglia of *Branchipus*. Surface view. Cephalic side at the top. (*After Hilton.*)

ment is connected with its neighbor in the same segment by transverse commissures, and with the ganglion of the next preceding and next succeeding segments by longitudinal connectives.

In Figs. 131, 132, and 133 Hilton's findings concerning the relations of cells and fiber tracts in some of the different ganglia of Branchipus are represented. Cells of the unipolar and bipolar types are common; multipolar cells are less numerous. In the ventral ganglia the cells tend to be massed into rather large ventrolateral groups, and smaller mesioventral groups. Many of the cells show a definitely fibrillar structure.

Some of the cells appear to have no large branches, but fibrillæ leave and enter the cell. The numbers of cells vary in the ganglia of different animals, and in different ganglia of the same individual. In Branchipus there are from 130 to 204 cells in each of the ventral ganglia. Cells also occur along the commissures, but their number varies widely from 35 or more down to 10 or less.

Hilton found that the brain of Branchipus is united from side to side by four commissures, a dorsal, two medial, and a ventral.

The ventral commissure lies below a small group of medial cells. It is in part made up of the processes of nearby cells and from the basal parts of the connectives. Many fibers of the medial tracts may be traced into the optic lobes. Fibers from the largest median cephalic cells descend the connectives. Fibers from cephalic and lateral cells groups cross in the center of the brain and either run straight into commissures or cross somewhat diagonally. Fibers from the smaller cells groups on the connectives near antennal nerves descend the connectives and ascend into the central parts of the brain to the same side or the opposite side. The small cephalic branches of the brain send fibers for a short distance into the brain and cells near here supply them. The mass of the connective fibers runs straight into the central parts of the brain. Fibers from cephalic lateral cells cross at angles to relate themselves to various cell groups, to run in the optic nerves, and to run into the central part of the brain.

In general, the distribution of tracts in the ventral ganglia is as follows:

1. Fibers in the connectives ascending or descending.

2. Fibers from the branches or nerve trunks end, cross in commissures, and ascend or descend in the connectives. Many end where they enter the ganglia, or on the opposite side in the same ganglion, or in the opposite ganglion.

3. Fibers in the commissures cross from cells of either group and end in relation to cells of either group of the opposite ganglion. Fibers in the commissures may also be seen to ascend or descend in the direction of the connectives.

4. Each cell area of each ganglion is probably connected as follows: (*a*) Fibers to other cell areas of each side through the commissures. (*b*) Fibers to cell areas of each side not from the other side through the commissures. (*c*) Ascending fibers. (*d*) Descending fibers.

The commissures are probably made up as follows: (*a*) Fibers from cells in upper levels. (*b*) Fibers from lower levels. (*c*) From the same level from both sides, especially from median cells. (*d*) Probably, fibers to and from lateral branches of ganglia, although this was not clearly demonstrated.

The form Corydalis is chosen because its nervous system is of a very generalized type. A pair of ganglia exists for practically every segment of the body. Only in the last abdominal segment has there occurred any of the condensation of ganglia which is characteristic of the higher insecta. The nervous system is distinctly of the ladder type of structure, as was that of Branchipus. The various ganglia of the central nervous system are connected longitudinally by means of longitudinal connectives. Cross-connections between the two sides of the nerve

cord are effected by means of commissural fibers. The nervous system consists of a pair of supraœsophageal ganglia, or brain, from which arise optic, antennal, clypeolabral, and other nerves, and a ventral nerve chain. The brain is connected to the ventral nerve chain by means of the crura cerebri. In this form the ventral nerve cord is made up of the subœsophageal ganglion, three thoracic ganglia, and eight abdominal ganglia. From the subœsophageal ganglia, the mandibular, maxillary, labial,

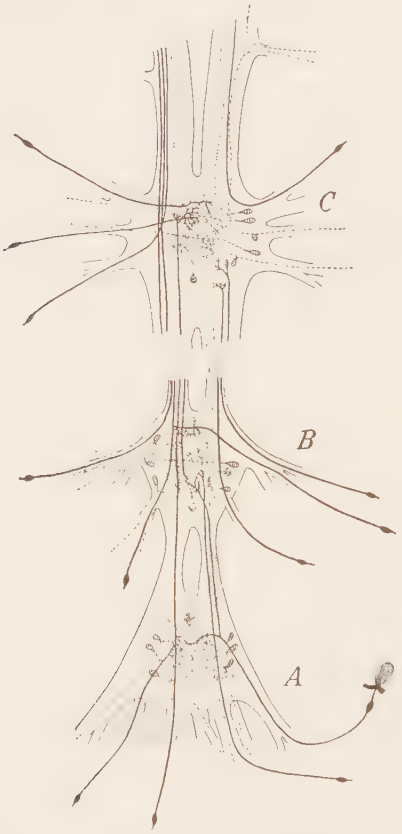


FIG. 134.—*A*, last abdominal ganglion of *Corydalis cornuta; B*, seventh abdominal ganglion; *C*, one of the thoracic ganglia. Solid lines———— indicate sensory nerve fibers; dash lines - - - - - - - - - indicate motor nerve fibers; dotted lines . . . . . indicate association nerve fibers. The various pathways should be followed in connection with the reading of the text.

gustatory, salivary, ventral, and median nerves arise which are associated with the sympathetic system. From the thoracic ganglia, nerves arise which go to the rear of the head, to the legs, to ventral and lateral muscles of the thorax, to the wing muscles of the adult, and to the tracheal system. From the abdominal ganglia, trunks which divide into five branches arise, supplying the abdominal muscles, tracheal wall, etc.

In addition to the ventral nerve cord, there is a sympathetic system which is divided into two parts—an upper and a lower. The upper sympathetic nerve is confined to the anterior and dorsal part of the alimentary canal, especially to the pharynx and œsophagus within the head. It is made up of an unpaired median or *vagus* portion and a lateral paired portion. The vagus nerve consists of a number of nerves—

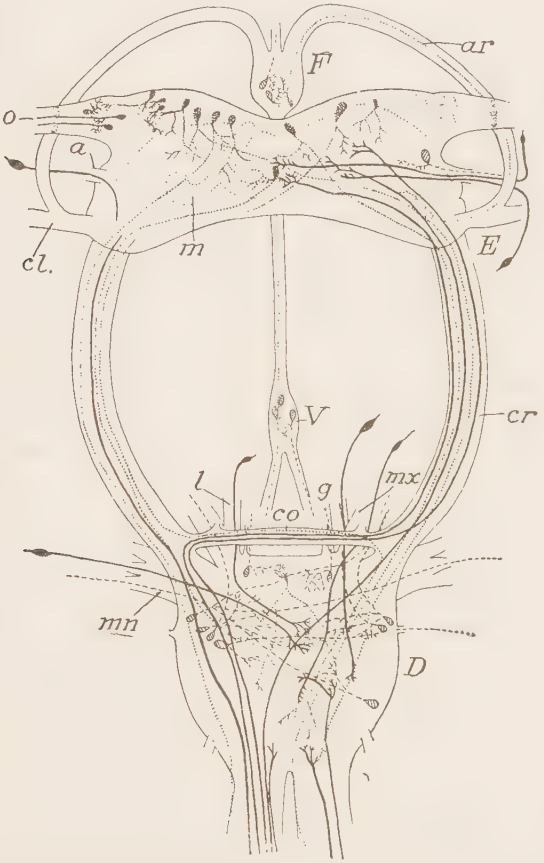


FIG. 135.—*D*, subœsophageal ganglion of *Corydalis cornuta; E*, Supraœsophageal ganglion; *F*, frontal ganglion; *V*, vagus ganglion; *a*, antennal nerve; *ar*, arched nerve; *cl*, clypeo-labral nerve; *cr*, crura cerebri; *co*, commissure; *g*, gustatory nerve; *l*, labial nerve; *m*, mushroom body; *mn*, mandibular nerve; *mx*, maxillary nerve; *o*, optic nerve.

pharyngeal, vagus ganglion, stomatogastric, recurrent, etc. The lateral or paired portion of this system is made up of small ganglia and nerves supplying the dorsal and lateral portions of the anterior portion of the œsophagus. The lower or ventral sympathetic system consists of a median, minute nerve extending backward from each of the first eleven ganglia. It usually gives rise to lateral branches connected with the respiratory apparatus.

Hilton has studied by means of intravitam methylene blue preparations and by serial sections the cellular arrangements of the central nervous system of Corydalis. Some of the results of his work may be seen in Figs. 134 and 135. In these figures the solid lines represent fibers of the sensory tracts, the dash lines represent fibers of the motor tracts, and the dotted lines represent fibers of the internuncial neurones. The central portion of the ventral ganglion is, in each case, filled with a felted neuropile in which numerous nerve fibers intermingle and end, while the motor and associational cells, as a rule (except in the supraœsophageal ganglia), lie ventrally and caudally to the neuropile. Hilton made out the following sensory tracts:

1. Fibers which end in the ganglia to which their nerve trunks are connected. These may end either on the same side or on the opposite side, or the fibers may divide and end on both sides of the cord.

2. Fibers which do not terminate in the ganglion to which their trunks are connected. Some of these run up the connectives to terminate in the ganglion next above, either on the same side, or the opposite side, or both.

3. Fibers which enter a ganglion, give off branches to its central mass, and then pass up into the ganglion next above to terminate there.

4. Fibers which enter a ganglion, and pass through more than one ganglionic center on their way toward the brain. Just how far a fiber mass may pass in this way was not determined.

The distribution of fibers in the larger head ganglia was much more difficult to determine. The following kinds were distinguished:

1. Fibers from connectives below, terminating in the ganglia, both on the same side as the entrance, and on the opposite side.

2. Fibers from various nerve trunks, terminating in the ganglia, usually in the deep mass of *Punktsubstanz*.

3. Fibers running from the subœsophageal up into the supraœsophageal ganglion, crossing in the commissure and running up the crus of the opposite side.

4. Similar to 3, but running up the same side. There are several groups of these fibers.

Hilton finds that most of the cells within the ganglia may be spoken of as *motor* or *association* cells. Usually, the cells are located in the posterior and ventral portions of the ganglia, and extend forward along the sides of the *Punktsubstanz*. The supraœsophageal ganglion has cells thickly massed on all sides except lateral and ventral. Processes from the cells penetrate the *Punktsubstanz*, and some of the more superficial cells send processes out over the surface of the ganglion. In the subœsophageal ganglion the cells form quite a covering on all sides, but especially at the caudal and cephalic regions.

Most of the motor neurones appear to send their fibers into the central *Punktsubstanz*. In this mass it is difficult to follow individual fibers with certainty, but it seems that many motor fibers emerging in the nerve trunks have their cell bodies on the opposite side of the cord. The motor cells are provided with numerous dendrites, and frequently lie at some little distance from the trunk along which their main processes pass. A smaller number of nerve cells each send one process directly into the nerve trunks nearest to them, and numerous dendrites into the *Punktsubstanz*. Motor tracts between the ganglia are not easily recognized, but there are indications that both ascending and descending motor fibers may pass through one or more centers.

All of the ganglia show a few cells with nerve processes which cannot be traced into any nerve trunk. These are called *association* cells. They may be small, but frequently are large. Some send their processes to the surface of the ganglion, apparently forming relations with many surface cells, while others send their processes straight down so as to bring into association dorsal and ventral neurones. Some cells send processes into both directions to ganglia above and below, and thus bring these into correlation.

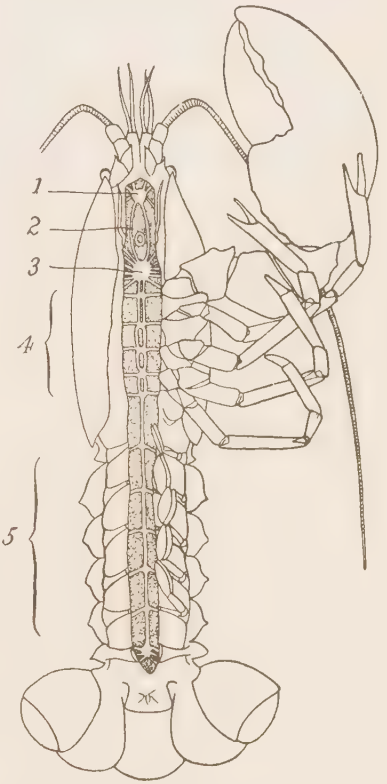


FIG. 136.—Central nervous system of the crayfish: 1, supraœsophageal ganglion; 2, circumœsophageal connectives; 3, infraœsophageal ganglion; 4, thoracic ganglia; 5, abdominal ganglia. (*From Loeb, "The Comparative Physiology of the Brain." By permission of G. P. Putnam's Son and Company.*)

The Nervous System of the Crayfish.—In the nervous system of the higher Crustacea, the processes of centralization and cephalization have gone on to a considerable extent. The nervous system of the crayfish (Fig. 136) may be taken as an example. In this form there are represented in the supraœsophageal ganglion the ganglia of three segments; in the subœsophageal ganglion, the ganglia of six segments; while the five thoracic ganglia and the six abdominal ganglia each represent a single ganglion.

Experiments which have been made upon the crayfish are very significant for an understanding of the functions of the nervous systems of

such segmented forms. Bethe found that, if both œsophageal commissures are sectioned so as to cut off the supraœsophageal ganglion from the rest of the central nervous system, all spontaneous progressive movement is destroyed. If the animal is stimulated, it will move forward for a short distance, but does not continue progressive movements for any length of time. Animals which have been deprived of the supraœsophageal ganglia show marked pendulum movements of the appendages, and these are unceasingly cleaning each other. The legs are somewhat more strongly flexed than normal at the junction with the body, due apparently to the fact that the extensor muscles have suffered loss of tension. The presence of the pendulum and cleaning movements in the operated animals, and their absence in the normal individuals, suggest that the supraœsophageal ganglion acts as an inhibitor to reflexes which would otherwise continually be taking place as the result of the stimulation of end organs connected with the segmental ganglia. It is also true that the operated animals are not able to grasp as strongly with their chelæ as do the normal animals. The destruction of the connection between the supra- and infraœsophageal ganglia does not interfere with the normal eating movements of the animals operated upon. Loeb, in his "Comparative Physiology of the Brain," quoted from Bethe the following statement:

> The animal devoid of the supraœsophageal ganglion is able to eat and selects its food. It is true that pebbles, small pieces of wood, etc., are seized by the forceps of the front pair of legs, but when brought near the mouth they are rejected. A piece of meat, however, is always taken into the mouth and masticated. The swallowing is difficult, just as in Carcinus. The piece often remains for a long time between the maxillipeds without being swallowed and at last falls to the ground. Pieces of paper that have been saturated with meat juice are treated in the same way. Stones that have been covered with meat juice are also brought to the mouth, but no attempt is made to masticate them. They are usually dropped as soon as they come into contact with the maxillipeds.

Among the Crustacea generally, it is true that cutting the longitudinal connectives between the subœsophageal ganglion, which supplies the mouth parts, and the first thoracic ganglion, which supplies the chelæ, has no serious effect upon movements of the locomotor appendages. In the crayfish, however, the locomotor movements are rendered impossible, even though the legs are not paralyzed, as is shown by the fact that they perform cleaning movements.

If, instead of cutting both circumœsophageal connectives in the crayfish, only the one on the right side is cut, there is a very marked difference in behavior. Bethe found that:

1. Stimulation of the left side of the head through contact induces movements of the appendages of the same side toward the stimulated area, followed by similar movements of the appendages of the left side.

2. The animal attempts to escape by a backward movement.

If the right side of the head is stimulated in a similar way, there is no attempt on the part of the animal to make such movements as have been indicated. Conduction posteriorly is limited to the longitudinal connective of the side stimulated. Transverse commissures are present in the supraœsophageal ganglia, but they are apparently not effective in calling forth bilateral reactions when the animals are stimulated in the manner suggested. While it has not been demonstrated, in so far as the author is aware, that the giant fibers (Fig. 126), which are of common occurrence in the Crustacea, have the same function as is assigned to them among the worms, it may be said that the presence of such fibers in the nervous sytems of the Crustacea makes understandable the very rapid and powerful movements involved in the backward swimming of the crayfish.

Cutting of one of the circumœsophageal connectives results also in a loss of one of the body muscles of the same side, and the body tends to become convex in outline on that side. Whenever the animal moves, it tends to perform circus movements directed toward the uninjured side.

Experiments made by Matula upon Sicyonia, a small decapod Crustacean, may be of interest at this point. An attempt was made to find the part played by the higher ganglia in the regulation of the reflex swimming movements of the animal. The nervous system consists of a cerebral ganglion, connected by two commissures with a chain of thoracic and six abdominal ganglia. The body wall is transparent and permits easy severance by means of a needle of the circumœsophageal connectives, or of the connectives between the thoracic and abdominal ganglia respectively.

The normal animal exhibits a forward swimming movement in which there is involved the rhythmic action of five pairs of pleopods, and a backward *flight* movement due to powerful flexion and extension of the abdomen with quiescent pleopods. After section of the longitudinal commissure between the thoracic and abdominal ganglia, mechanical stimulation caused, in 70 per cent of the animals, a reflex movement of the last pair of pleopods alone; while in a few cases the higher abdominal segments were excited in diminished degree. After section of the circumœsophageal commissure each pleopod responded to slight stimulation, while stronger stimulation initiated rhythmic swimming movements instead of the normal response of the backward flight. Matula concluded that the centers for coordination of the pleopods lie in the abdominal ganglia, but that their excitability is small when they are separated from the thoracic ganglia. When the thoracic and abdominal ganglia are intact, reflex excitability is increased, but the action of the pleopods can be inhibited only when the whole nervous system is intact.

The Nervous System of Limulus.—The horseshoe, or king crab, Limulus, is provided with a very elaborate, complicated, and highly centralized nervous system. This nervous system has the segmental arrangement characteristic of other Arthropoda, as shown in Fig. 137. This figure is copied from the drawing by Patten and Redenbaugh in the *Journal of Morphology* and gives some idea of the complexity of struc-

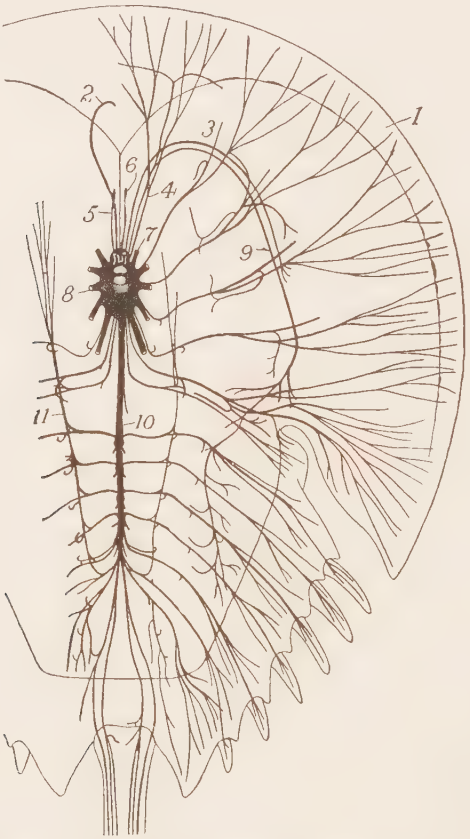


FIG. 137.—The nervous system of *Limulus polyphemus:* 1, carapace; 2, median eye nerve; 3, hæmal branch of integumentary nerve; 4, second hæmal nerve; 5, right olfactory nerve; 6, median olfactory nerve; 7, supraœsophageal ganglion; 8, origin of fourth neural nerve; 9, lateral nerve; 10, ventral cord; 11, sympathetic nerve. (*From Patten and Redenbaugh.*)

ture found in the ring about the œsophagus. The animal is provided with a variety of sensory receptors, including eyes, gustatory organs, endings of temperature sense, etc. There are also developed in the nervous system definite centers of control of various functions—visual, gustatory, cardiac, respiratory, etc. In this animal it is possible, on the whole, to study to good advantage the normal behavior, the reactions to careful and well-controlled stimulation, the effect of operations, etc.,

since the animal is somewhat sluggish and does not become greatly excited under laboratory conditions, if it is carefully handled. The greatest difficulty experienced in working with this form is due to the almost unavoidable loss of blood as the result of operating upon the central nervous system.

Studies of the functions of the various parts of the central nervous system have been made by Loeb, Miss Hyde, Patten, and Pearl. The central nervous system consists of supraœsophageal or cerebral ganglia, an œsophageal ring, a subœsophageal ganglion, and a series of abdominal ganglia. These structures are evidently not perfectly homologous with structures bearing the same names which are found in other Arthropoda. For example, the œsophageal ring is here a structure containing many ganglion cells instead of being simply a connective between ganglia. It is as if the connectives had become very much shortened, so as to bring some of the thoracic ganglia up very close to the supraœsophageal ganglia.

Experiments have shown that when the right half of the supraœsophageal ganglion is removed the animal tends to move in a circle toward the left. After a time these circus movements disappear. If the whole of the supraœsophageal ganglion is removed, the animal may take food which is placed upon the organs of the mouth, but it has lost all spontaneity of movement. It no longer recognizes members of the other sex.

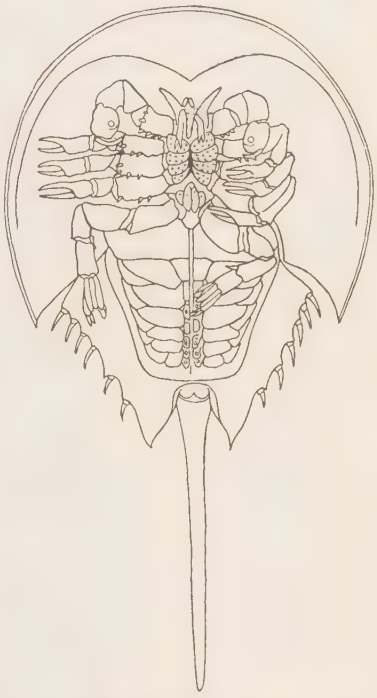


FIG. 138.—Diagram showing dominance of flexors over extensors in the thoracic appendages of *Limulus* in which the right half of the brain has been destroyed. (*From Loeb, "Comparative Physiology of the Brain." By permission of G. P. Putnam's Son and Company.*)

The centers controlling food-taking and eating movements are located in the œsophageal ring. If the ring is cut, circus movements toward the injured side take place. This is due to the natural dominance of flexors over extensors as shown in Fig. 138. If ganglia are removed from the œsophageal ring, permanent paralysis of the corresponding appendage results. Each appendage behaves normally so long as the portion of the œsophageal ring from which it receives its nerve supply is in normal relation with it. Feeding movements are reflexes involving chemical and contact receptors on the coxal portions of the appendages. Stimulation

of these brings on a muscular spasm of the stomodæum by which means food materials tucked into the mouth are swallowed. The leg movements employed in mastication involve both flexor and extensor muscles. Patten is of the opinion that the motor ganglion cells controlling the movement of the appendages lie in pedal ganglia which are located between the central nervous system and the bases of the appendages.

Removal of the subœsophageal ganglion destroys the power of the animal to move normally. The animal remains quiet, though it may carry on normal respiratory movements of the gill plates. In the normal animal a gentle stimulation of the outside of the thorax, as by warmth, causes aimless movements of the legs of the opposite side, followed by aimless movements of the legs on the same side. Stronger stimulation has the effect of causing the legs of the same side to make coordinated movements tending to thrust the stimulating object away. The legs of the opposite side may join in this effort. Here, we have a very good example of a crossed thoracic reflex.

If the nerve ring is cut on one side, stimulation of the cut side produces the same result as before, except that the legs back of the cut, on the same side, do not make purposeful "thrusting-away" movements, while those cephalad to the cut do make such movements if the sides of the thorax cephalad to the cut are stimulated.

In Fig. 137, showing the arrangement of the central nervous system of Limulus, fine cross-lines will be noted just back of the œsophagus. These represent thoracic transverse commissures. If all the transverse commissures be cut, all the crossed reflexes cease.

Control of the Respiratory Mechanism.—Loeb had claimed that the respiratory function is served by all the segmental abdominal ganglia, whose nerves pass to muscles activating the gill plates. Each ganglion was considered to have automatic activity and control over the gill plate of the corresponding segment. The rate of respiration is apparently determined by the temperature and the concentration of carbon dioxide in the blood. The gill plates move in unison so long as the abdominal nerve trunk is intact. If abdominal ganglia are isolated from each other, the respiratory rate is maintained by all the gill plates with a fair degree of constancy, but the phase of movement may not be the same in all appendages. Loeb expressed the opinion that the ganglion which acts most quickly to the natural stimulus stimulates those ganglia connected with it and so determines the correspondence of phase. When the connection between adjacent ganglia is broken there is no longer the possibility of such correlation. Patten claims that only a part of the respiratory mechanism is located in the abdominal ganglia, and that a very important part is located in what he calls the vagus ganglion, a ganglion lying at the posterior end of the subœsophageal ganglion.

Nervous System of the Honeybee.—For a statement as to the structure of the nervous system of the honeybee, the author is indebted to the work of Dr. Snodgrass, three of whose figures are here reproduced. As

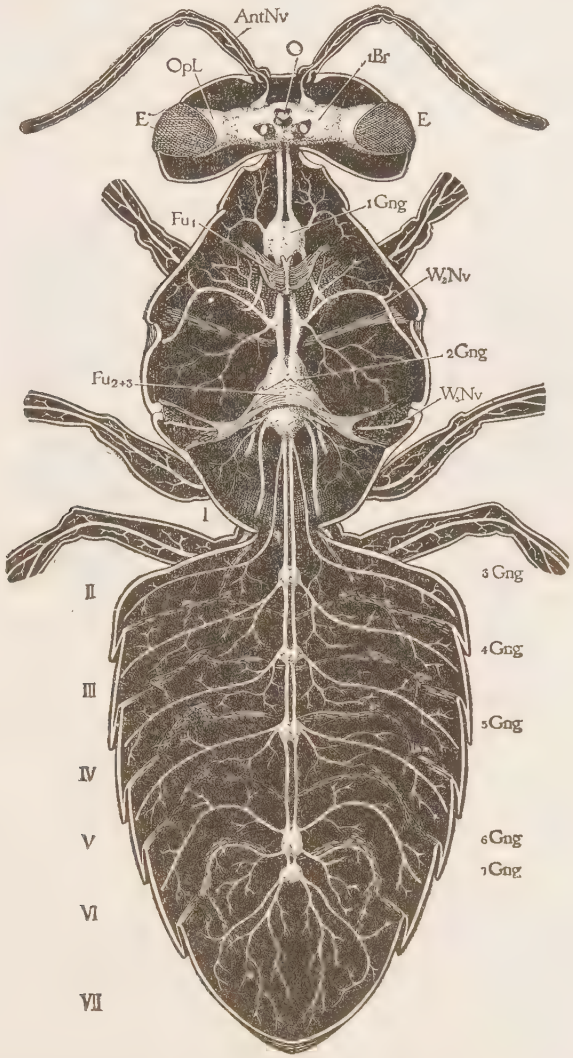


FIG. 139.—Nervous system of the worker bee: AntNv, antennal nerve; O, ocellus; E, compound eye; 1Br, protocerebral lobe; OpL, optic lobe; 1Gng, 2Gng, thoracic ganglia; 3Gng–7Gng, abdominal ganglia. (*From Snodgrass, "Anatomy and Physiology of the Honeybee."*)

will be seen in Fig. 139, the nervous system of the honeybee shows the same fundamental ladder type of structure and the segmental features that have been found to characterize the nervous systems of other arthropods and worms. It will be noted that there is, in proportion to the size

of the animal, a greater development of the nervous system than is found in many other arthropods. There is also evidence of very great centralization and cephalization of nervous elements (Figs. 140 and 141). Embryological study has shown that there are three pairs of ganglia represented in the supraœsophageal ganglia, and three pairs in the subœsophageal ganglia. These ganglia are very closely appressed, and the circumœsophageal commissures are so shortened that the subœsophageal

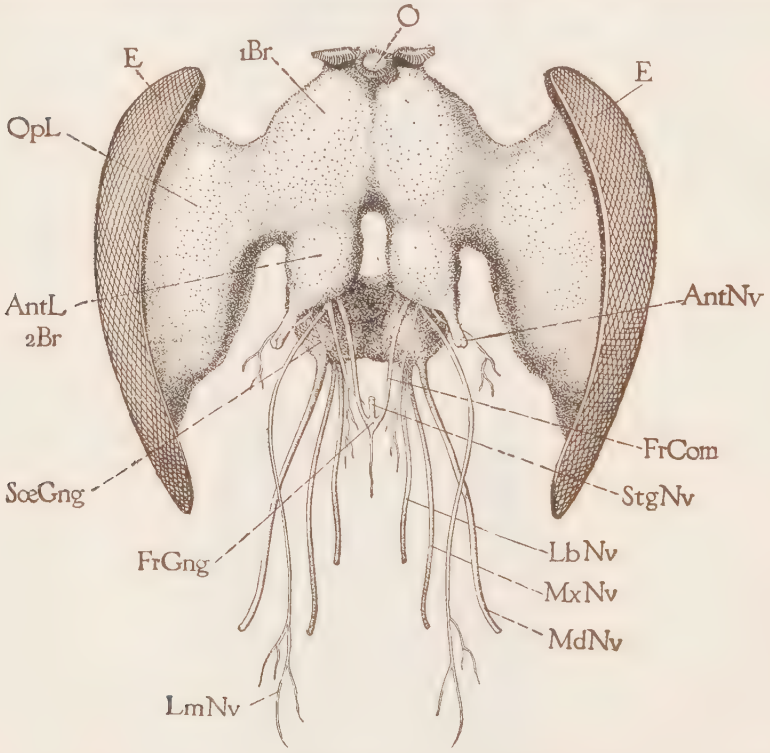


Fig. 140.—Brain and subœsophageal ganglion of worker bee, anterior view: O, ocellus; 1Br, protocerebral lobe; AntL, antennal lobe; AntN, antennal nerve; FrCom, frontal commissure; LbNv, labial nerve; MdNv, mandibular nerve; E, compound eyes; OpL, optic lobe; 2Br, deutocerebral lobe; SoeGng, subœsophageal ganglion; StgNr, stomato-gastric nerve; MxNv, Maxillary nerve. (*From Snodgrass, "Anatomy and Physiology of the Honey bee."*)

ganglia appear to be almost directly attached to the supraœsophageal ganglia. The ventral nerve trunk consists of seven ganglia, two in the thorax and five in the abdomen. The second thoracic ganglion is very clearly a compound ganglion, being made up of the ganglia of four segments. It innervates both pairs of wings, the middle and third pairs of legs, the muscles of the mesothorax and metathorax, the propodeum, and the first segment of the abdomen. The first and second ganglia of the abdomen lie in the second and third segments, but they really belong in

the third and fourth abdominal segments. The fifth, sixth, and seventh abdominal ganglia lie in the segments they innervate, and belong to them. The seventh ganglion is composed of four ganglia and belongs to and innervates segments seven, eight, nine, and ten.

In the bee it is found again that the ganglion of each segment controls directly the activity of the segment. The head of the animal may be removed without destroying locomotor movements, though such movements may be rendered very awkward and uncertain. There is an indication here of the importance of the head ganglia as coordinating structures.

The great development of the head ganglia is associated with an enormous development of the head-receptor system. The simple eyes, compound eyes, antennæ with their eight varieties of sense organs, such as sensory hairs and sensory plates, Johnston's organ, etc., giving information as to touch, taste, smell, etc., bring to the head ganglia a flood of afferent impulses which it is the business of these ganglia to interpret

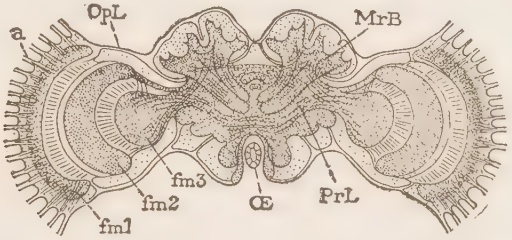


FIG. 141.—Vertical cross-section of the brain of worker bee. Dark parts represent fibrillar masses; outer light parts, cellular tissue: a, fringe of subretinal nerve ends; fm_1, fm_2, fm_3, outer, middle, and inner fibrillar masses of optic lobe; MrB, mushroom body; OpL, optic lobe; Oe, œsophagus; PrL, protocerebral lobe. (*From Snodgrass, "Anatomy and Physiology of the Honeybee, after Jonescu.*)

and mediate. It is doubtless true also that very many sensory impulses from other regions of the body are relayed up through the ventral cord and connectives to the cerebral ganglia. In the brains of bees there are developed very prominent structures known as *mushroom bodies*, which seem to have been accepted by zoölogists as centers of general brain activities, especially those which have been termed mental activities, or perhaps better referred to as instinctive activities, and those activities which involve memory. Great masses of both sensory and motor fibers center in these mushroom bodies. Jonescu claims that the mushroom bodies must be the place in the central nervous system where the various sense impressions are recorded, where actions are coordinated, and where associations are established and retained, in which sense they may be considered as organs of intelligence. No such organs are found in the other forms whose nervous systems have been considered here.

CHAPTER XXIX

PHYSIOLOGY OF REPRODUCTION

The phenomena associated with the processes of reproduction have in them so much of mystery and interest that the real significance of the process is likely to be missed. It is only natural, it may be supposed, to think of the process of reproduction as a process having for its object the production of new individuals of the same kind as those which have preceded it. There is a tendency to think of individuals as being of great importance, while, as a matter of fact, it is more probable that it is the race that is, after all, of supreme importance, and that the individuals are only incidents in the racial history. The race of organisms must not be thought too much as something set apart and different from other races, though this may be true; but the race should be thought of as representing an organic continuity reaching back for millions of years to conditions, the simplicity of which can only be guessed, and potentially reaching forward to conditions of complexity and high organization, concerning the exact details of which biologists are in no position even to speculate. Now, also the fact, mentioned very early in this work, must be called to mind that the organism, whether it be a single cell or a more complex multicellular aggregate, is, after all, a complicated physicochemical system. The thing which the organism provides for is, then, the continuity of the particular physicochemical organization of which it is a part.

The methods by which various types of organisms undertake to provide for this organic continuity show a wonderful evolution from the simple processes of budding and fission, seen in the very simple forms of animals, up to the highly complicated sexual reproductions seen in the mammals. These differences in the reproductive processes are not to be thought of so much as differences of kind, however, as differences of degree, in which the details of the reproductive process have been improved, and differences enforced by the varying environmental conditions under which development takes place.

Biogenesis.—Two terms which have held prominent place in biological literature for the past seventy-five years are *biogenesis* and *abiogenesis*, the former referring to the fact that living matter arises only from living matter, and the latter to the possibility that living matter may arise from non-living matter. While the experiments of Pasteur, Tyndall, and others appear to have disposed of the possibility of the origination of

living matter from non-living matter under the conditions prevailing in the particular experiments mentioned, the facts presented, however, do not render impossible the origination of living matter from non-living matter at some period of the earth's history or under conditions then existing, and which may still exist. The facts that living stuff is made of the same elementary substances as non-living matter, and operates in accordance with the same laws, certainly lend weight to the suggestion that the associations of these elements into the particular unstable compounds which are recognized as characteristic of living beings form a definite and logical step in the evolution in the higher types of matter from the simpler.

The significance of the colloidal condition, and the possibility that inorganic colloids may have played an important part in the synthesis of some of the simpler substances which have long been considered to be the results of organic action only, have only recently been recognized. The work of Benjamin Moore clearly shows the possibility of the synthesis of simple carbon compounds from carbon dioxide and water through the use of inorganic colloids as catalysts.

Fundamental Types of Reproduction.—It has been customary on the part of biologists to speak of two main types of reproduction among animals, asexual and sexual. As a matter of fact, it is very difficult to draw a sharp-line distinction between these two types because of the way in which they intergrade. It has also been customary to subdivide those asexual processes which do not involve any union of gametes, or sexual cells, into a number of different forms:

a. Budding, or Gemmation.—This is a form of asexual reproduction in which the new organism develops as a bud from some part of the body of the parent. The buds may remain attached to the parent, in which case compound animals, or colonies, are formed. Such budding occurs in corals, hydroids, polyzoans, compound Ascidians, etc. In some forms the buds become separated from the parent, as in Hydra. In some cases the term is extended to include the formation of new individuals by a similar process within the body of the parent. In unicellular organisms, as the yeast plant, gemmation is simply a form of cell division in which one of the new cells is much smaller than the other.

b. Fission.—This is a form of reproduction in which an animal body spontaneously divides into two parts, each of which grows into a complete organism. In unicellular organisms, it is identical with cell division.

c. Sporulation, or Endogenous Cell Division.—Reproduction of this sort occurs among many of the protozoa. A cell forms a cyst with a thick wall, in which the protoplasm of the cell divides repeatedly, forming many cells. Under appropriate conditions, the cyst is broken and the many daughter cells are released.

Sexual Reproduction.—There can be little doubt that the earlier forms of the reproductive process found among animals showed little or no evidence of sex differentiation. In most animal groups, some form of sexual reproduction has developed in which two sexual *gametes*, a *macrogamete*, or egg cell, and a *microgamete*, or sperm cell, are brought together through a definite process of conjugation into a common *zygote*. Such conjugations are now known to occur in all animal groups from the protozoa up. It appears to be true that, if a protoplasmic line is to be continued for a long time, it must seek its perpetuation through combination with some other similar line. Whether the rejuvenation of the germinal material was the end sought in the first attempts at conjugation of germinal cells may be a matter of doubt, though there can hardly be doubt as to the end result. Certainly, the admixture of the different lines had the effect of providing for variation in the character of the resulting line, and with variation there would be the possibility of what has come to be called evolution.

Reproduction among the Metazoa. A Series of Phenomena.—Reproduction among the metazoa is to be looked upon as a series of processes which are so closely interrelated as to seem to be almost hopelessly scrambled together. Careful analysis has shown, however, that it is possible to separate one process from another, and so come to a clearer understanding of the whole complicated physiological reaction.

The following diagrammatic scheme indicates some of the larger steps or phases of the reproductive process:

Differentiation and growth of germ cells
Maturation of egg and sperm cells
Ovulation
Insemination
Fertilization:
  External
  Internal—copulation
Cleavage
Implantation
Placentation
Organ formation
Fœtal-membrane formation

The Egg Cell a Highly Specialized Cell.—The statement has sometimes been made that the egg cell may be taken as an example of an undifferentiated or unspecialized cell. Our present understanding of the egg cell and its activities indicates that the egg is one of the most highly specialized cells in existence. As a protoplasmic cell, it exhibits the same fundamental properties as pertain to other protoplasmic cells. Its property of irritability seems to be somewhat specialized and limited, in that it responds to the stimulus furnished by a spermatozoön of its

own kind with a remarkable display of energetics, while it does not ordinarily respond in the same way to other forms of stimulation. In order to do its work as an egg cell, and finally give rise to an organism capable of independent existence, it must be provided with:

1. A mechanism of cell division. This mechanism must be of a sort such as will provide for the proper division and distribution to the daughter cells of both nuclear and cytoplasmic materials. It must, therefore, be highly organized.

2. Diversity of structure and composition. The egg cell must have the power to produce cells of as many kinds as are to be found in the adult organism. This does not mean that the egg cell shall divide at once into muscle cells, nerve cells, gland cells, and the like, but that there must be in the egg the potentiality for differentiation, and a definite physicochemical architecture.

3. Large supplies of energy-furnishing material. The egg must have the power to divide itself into many cells. This work calls for the expenditure of energy. The material for supplying this energy must be present in the egg to carry it along in its early developmental history to a time when it may be able to take in food materials from without.

4. A high degree of sensitivity to changes in external conditions. The development of the egg must take place under certain definite and highly stable environmental conditions. It must possess, therefore, a highly responsive physicochemical balance. As the egg is a colloidal system, this prerequisite is provided for. The development of this unstable system is, then, a series of interactions between the egg and the environment in which it is placed.

5. The ability to develop into a definite and specific organism. This specificity is undoubtedly fundamentally a matter of chemical constitution and arrangement. It has already been seen that specificity in the adult is a matter of chemistry. It certainly cannot be less so in the embryo or the egg.

The degree of success which may be attained by a germ cell, in playing its part in forming an adult of any given species, may be measured by the exactness with which it approaches the chemical nature of the line which it is supposed to represent, and by the exactness with which it is adapted to the conditions of the environment in which it passes its developmental stages.

The egg cell is frequently looked upon as a storehouse of heritable characters received from the two parents. It is more than this: It is a mechanism for cell multiplication, upon which is imposed a mechanism for assorting and properly distributing to the daughter cells those hereditary determiners received from the two parents, and a mechanism for developing, from an apparently homogeneous egg protoplasm, a highly heterogeneous adult form.

The Germ Cells.—Very early in the life history of any individual there are set aside certain cells which, because of the part they are later to play, are known as germinal cells. When these cells are first set apart, it is not possible to distinguish whether they are to develop into ova —egg cells—or spermatozoa— sperm cells. As the adult life of the individual approaches, these cells of the germinal epithelium give rise to highly differentiated sperm or egg cells.

The fact that the cell is to be considered a physicochemical system has already been emphasized. This idea must now be carried over to the structure and functions of the germ cells. The germ cells, either egg cell or sperm cell, must be thought of as a physicochemical system, colloidal in character, produced within the body of the living organism, under the influence of a definite organic environment, and coming to have a peculiar and definite physicochemical architecture and balance, such as to render it able to convey to its descendants those physical and chemical qualities and arrangements which characterize the organism from which it was derived. It is not enough to think of the cell as a whole as being a physicochemical system, but the idea must be accepted, that, even in its most finely divided parts, such as nucleus, chromosomes, etc., it is such a system. The fact must also be kept in mind that not only is the cell such a system, but that it has its peculiar properties because it is the result of the division of a previously existing similar system, and that it may in time itself give rise, by division, to daughter systems similar to itself. It must be recognized that the cell is what it is because of the chemical composition and physical arrangements of all its parts, *i.e.*, its physicochemical configuration. If the configuration is modified, the cell is modified.

A study of the life histories of the ova and spermatozoa reveals the fact that these two sets of cells pass through developmental cycles which are practically parallel. In each case the germ cells are derived from primordial germ cells, which divide many times during the early life of the individual. This is the period of multiplication. It is during this period that the vast numbers of very minute oögonia and spermatogonia are formed which may later in the life of the individual give rise to the functional germ cells. In any organism not all of the cells formed during this period of multiplication are likely to function as germ cells. Some will be lost even though they become mature. Some will not pass through all of the phases necessary for the full maturing of the germ cells. Some may be absorbed and their material used by other more active germ cells. Some will eventually become mature and function as reproductive cells.

The second period in the development of the germ cells is a period of rest and growth. It is during this period that it becomes possible to distinguish spermatogonial and oögonial cells from each other. Those

cells which are to become ova accumulate large amounts of food materials. The non-living matter in these cells may become many times more in bulk than the living matter. The cells grow rapidly in size. The substances laid down in the ova during this period include food yolk, of which there may be a vast amount in proportion to the amount of active protoplasm, and substances of the general nature of hormones or autocoids, or of substances from which such hormones may be derived by the metabolic processes of the egg. These hormone substances are later to play an important part in regulating the activity and direction of developmental processes in different regions of the embryonic body.

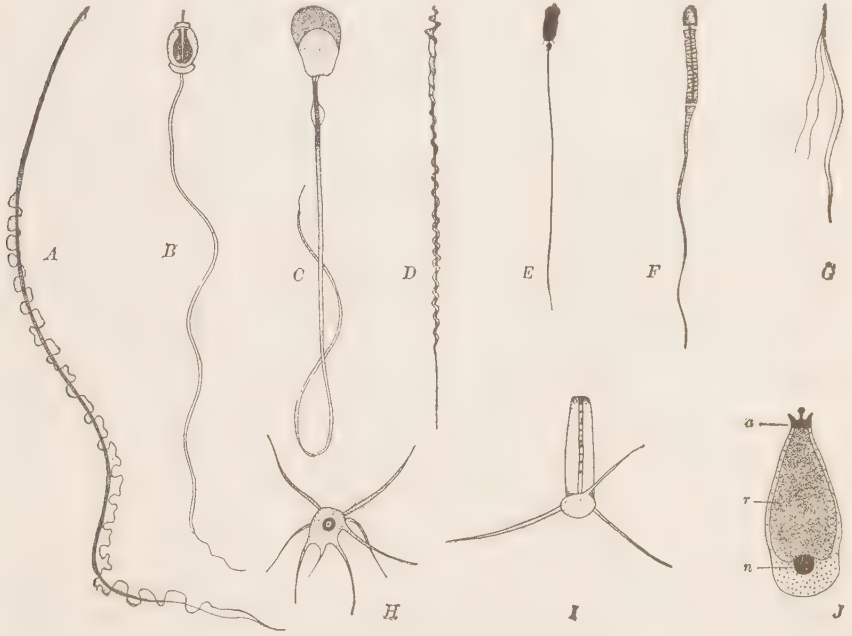


FIG. 142.—Various types of spermatozoa: *A*, *Triton* (salamander) (*after Ballowiiz*); *B*, *Nereis* (annelid) (*after Lillie*, 1912); *C*, Guinea pig (*after Neves*); *D*, Phyllopneuste (bird) (*after Ballowitz*); *E*, Sturgeon (*after Ballowitz*); *F*, *Vesperugo* (bat) (*after Ballowiiz*); *G*, *Castrada* hofmanni (turbellarian) (*after Luther*); *H*, *Pinnotheres veterum* (crustacean) (*after Koltsoff*); *I*, *Homarus* (lobster) (*after Herrick*); *J*. *Ascaris* (nematode); *a*, apical body; *n*, nucleus; *r*, "refractive body" (*after Scheben*).

In the cells which are destined to form spermatozoa, very little of the morphological change which is so characteristic in the oögonial cells is to be seen. These do not increase markedly in size. The cells are, however, becoming internally organized for the work which they are later to do.

The third phase of the development of the germ cells is the period of maturation. Up to this time the number of chromosomes in each

oögonial cell and in each spermatogonial cell has been the same as is found in the somatic cells of the individual, a number which may be designated by the symbol $s/1$. During the maturation phase, the number of chromosomes is reduced to one-half the somatic number (s 2) in preparation for the later fusion of the egg and sperm nuclei.

FIG. 143. Diagram of typical flagellate spermatozoon; P, perforatorium; A, acrosome; N, nucleus; M, middle piece; F, axial filament; S, cytoplasmic sheath; E, end piece. (*After Wilson.*)

In each primordial germ cell developing in ovary or testis, there is a complex of hereditary determiners, derived from paternal and maternal sources, and which comprise the chromosome complex of the nucleus. Each chromosome is bivalent in character, one-half having come from the paternal and one-half from the maternal side. In order that the number of chromosomes shall be maintained constant in any given species, it is essential that one-half of each bivalent chromosomal pair shall be discarded before the cell is to function in the process of fertilization. The details of the process belong more properly to a course in cytology than to a course in physiology, though the significance of the process is fundamental. It is essentially a casting aside—at least a throwing out of the nuclear mechanism—of chemical substances derived from ancestral cells, substances which, when present in the chromosomes of the germ cells, exert a directive or determining effect upon the destiny of the daughter cells. These substances are the hereditary determiners. Morphologically, they are in all probability contained in the minute jelly-like granules which comprise the chromosomes.

Forms of Mature Spermatozoa.—The mature spermatozoa of different animals vary much in size and form. Some of the diverse forms are shown in Figs. 142 and 143. Each spermatozoön consists of a head portion, middle piece, and tail. In addition, there may be other specialized portions, as perfertorium, acrosome, etc. The head of the spermatozoön contains the nucleus, and corresponds to the nucleus of the egg cell. It contains the chromatin, which serves as the carrier of the determiners in heredity. The head is usually surrounded by a thin layer of cytoplasm. The middle piece is a cytoplasmic structure located just back of the head, and contains the centrosome, chondriosomes, and other inclusions. The tail is made up of two portions, an axial portion which extends backward from the middle piece, and a sheath of cytoplasm which usually covers nearly the whole length of the axial portion. This sheath is variously modified

into undulating membranes of one form or another in some animal groups.

Egg and Sperm Shedding.—There is great variation in different groups of the animal kingdom as to the manner in which the male and female germ cells are brought into contact. Even in those forms in which there is no copulation, there are undoubtedly metabolic disturbances, and in many cases the shedding of eggs and of sperm is the result of stimuli of a chemical nature. Such is certainly true in the case of some of the Nereid worms in which individuals of each sex give off into the sea water some substance which stimulates individuals of the other sex to sex activity. The response is almost instantaneous and so very definite as to be very spectacular. Among the Echinoderms, the sperm is shed by the males, and within a few seconds females in the vicinity will begin to shed eggs. This, again, is probably to be interpreted as a response to chemical stimulation.

Among the higher animals, especially the mammals, the correlation of the activities of ovaries and accessory sex organs is affected by means of hormones carried in the blood stream.

H. Monroe Fox found that in the sea urchin, *Centrechinus setosum*, the ovaries and the testes show a remarkable periodicity of activity associated with the lunar period, the genital products being spawned into the sea about the period of the full moon during the breeding season. After spawning, the gonads become reduced in bulk, the declining phases of the moon being occupied by recuperation and the commencement of the preparation of fresh gonadal products. After the new moon, these increase in ripeness and in bulk, and, with this development, the size of genital glands grows visible once more.

Microscopic examination of the gonads of a number of individuals between the first quarter and the full moon shows the majority to be swollen and packed with fully formed eggs or spermatozoa. A small proportion are "spent," that is, they show evidence of having recently extruded their genital products. A week later the relative proportions are reversed, some individuals have gonads still full of spermatozoa or eggs, but most are spent. Between the third quarter and the new moon all gonads are shrunken in size and contain nothing but developing spermatocytes and oöcytes. From now onward, until the first quarter of the next moon, these cells show progressive stages in development into spermatozoa and eggs, destined to be spawned around about the time of the full moon. This lunar cycle is repeated throughout the breeding season.

The following data, taken from the article by H. M. Fox, indicate a very widespread condition of lunar influence, calling for a great deal of further study:

Species	Swarming Habit
Palolo worm (*Leodice viridis*) (Pacific)	Last quarter of moon in October and November.
Palolo worm *Leodice fucata* (Atlantic)	Third quarter of moon in June and July.
Palolo worm (*Ceratocephale Os*)	Full moon and new moon.
Palolo worm (*Staurocephalus greg.*)	Last quarter of moon, sometimes first quarter. Tide seems not to be the sole cause of swarming. Light appears to be essential.
Odontosyllis	First to third quarter of August moon.
Nereis limbata (Woods Hole)	June and September. Full moon. Sink to low point about third quarter, increase again and decrease to vanish at new moon.
Platynereis megalops (Woods Hole)	Waning phases of moon during summer months.
Nereis (Naples)	First and third quarters of moons of October to May.
Amphitrite	New and full moon, spring tides.
Convoluta	Only at neap tides.
Chiton	Full moon.
Fish *Leuresthes tenuis*	Second, third and fourth nights after full moons of March, April, May, June.

As to the physiological cause of such swarmings, perhaps the most that can be said is that the reaction is due to some influence which varies with the lunar periods, and which has affected the animals for a long time antecedent to the swarming period.

For in order that all the worms should be sexually mature and ready to swarm together at, say, the last quarter of a certain moon, the development of the genital organs must have been initiated in all of them at some definite previous lunar time.

The higher temperature of the sand flats upon which some of these swarming forms live, due to the abnormally low water at the spring tides, together with a more abundant food supply, is supposed by Scott to have a causal relation to this reproductive cycle.

Treadwell, in collaboration with Tashiro, has shown that the period of swarming of the Atlantic Palolo worm is preceded by a period of increased metabolic activity. This was demonstrated by means of the biometer, by which Tashiro was able to show that the carbon dioxide production ranged from:

0.000,000,000,07 grams carbon dioxide per minute 5 days before egg laying, and 0.000,000,000,083 grams carbon dioxide per minute 2 days before egg laying, to 0.000,000,000,13 grams carbon dioxide per minute during the swarming period.

The conclusion reached was that the increased metabolic activity of the animal, as the time of swarming approaches, furnishes an inner stimulus of importance in producing the swarm, and the suggestion is made that probably a similar stimulus is operative in ordinary egg laying.

Arrhenius has shown, through a statistical study of some 25,000 cases, that there is a lunar periodicity of human births. Also, he has shown through a study of 12,000 cases from maternity hospitals of Stockholm, that there is a lunar periodicity of the onset of menstruation among human females. At 18 days after the moon passes through the ecliptic from north to south, there is a minimum number (6.5 per cent fewer than the average for all the days of the lunar month), and then the number rises again to a maximum at another part of the month. It is pointed out that in each individual the onset of menstruation depends upon a number of physiological and psychological causes, which occur without any rhythm. In addition, there is a rhythmic causative factor having the period of the revolution of the moon. This factor is weak and will only make itself felt when acting in conjunction with the other causes. Menstruation may occur, then, at any time according to the resultant effect of all the causative forces acting upon the person, but in more instances than not it will fall in that part of the tropical lunar period in which the periodic factor is acting.

Arrhenius suggests, in regard to the human cycle, "that the factor in question is the amount of electricity in the atmosphere," which he has shown to vary rhythmically with a period of 27.32 days. But in addition to this period, the atmospheric electricity has a shorter period of 25.93 days (non-lunar). If, now, the same shorter period could be demonstrated in the menstruation figures, the possibility of a causal connection between the two phenomena would be heightened. Further investigation has shown that the shorter period is present in the case of menstruation. In addition, the effect of the two periodicities should be to cause the average time between menstrual periods to be 26.605 days. The average for 1834 individuals in Denmark was found to be 26.68 days.

Factors Involved in Development.—It will be recalled that, in our discussions of various tissues and organ system, it has been shown that the behavior of a tissue represents the interaction of both internal and external factors. Also, in the study of the nervous system, it has been pointed out that the behavior of an organism represents the resultant of all the forces, internal and external, which come to bear upon the mechanism called the body of the animal. Reproduction is now to be thought of as a type of behavior. In this case a fertilized egg cell or zygote—a physicochemical system—acting under the influence of a more or less definite environment is being dealt with. Also, the position that the fertilized egg is an organism must be taken into consideration. The influence of external factors is recognized as influencing the behavior of the adult organism. Here, it must be recognized that the work of cell multiplication, of differentiation, and of organ system building represent a normal part of the activities—the behavior—of the developing organism.

The processes of development have commonly been looked upon as the expressions of the inherent activity of the fertilized egg. The purpose here is to emphasize the view that development is really the resultant of the interaction between the fertilized egg and its environment. The factors involved in development are then:

1. *Internal,* contained within the egg:
2. *External,* forming the immediate environment of the egg.

Of the internal factors involved in development, but little is known. From what has been said, it seems safe to assume the existence of a colloidal mechanism adapted to growth and self-division, upon which is imposed a mechanism for the transfer of hereditary determiners from one cell generation to the next. In this colloidal complex, there seem to be provided, also, chemical agents of a hormone character which serve, like the hormones of the adult, as the regulators of chemical reactions taking place within the dividing cells.

A great variety of external factors may influence development. Among them may be mentioned:

1. Relative proportions of salts in the environing liquid.
2. Total concentration of salts (osmotic pressure) of the environing liquid.
3. Temperature.
4. Hydrogen-ion concentration.
5. Gravity.
6. Electricity.
7. Light.
8. Mechanical agents.
9. Oxygen supply.

What is called normal development (meaning most usual), depends very definitely upon the maintenance of the external factors at what may be termed optimum values. Slight variations of any of the conditions mentioned have the effect of making profound changes in the course of development for the reason that they interfere in one way or another with the rate or the proper sequence of the chemical changes involved in cell multiplication or cell differentiation.

The conception of the egg cell as a highly complex physicochemical system, now has added to it the idea that this system can go through the series of orderly chemical changes which would lead to the formation of a normal adult organism only when the egg is provided with an environment of a particular chemical and physical constitution, and with which it is free to react.

The fact that certain chemical changes have taken place in the developing egg is indicated by the form which the embryo assumes. That is, the form of the developing individual is an indicator that a certain portion of the normal sequence of chemical operations charac-

teristic of the species has occurred. Developmental stages are, then, simply indications that certain definite portions of the chemical history of the individual have been passed through.

Fertilization.—It has commonly been stated that the egg cell is a passive sort of thing and that the spermatozoön is highly dynamic. This statement cannot be accepted as being wholly true. The egg cell must be thought of as being potentially the storehouse of all those characters, physical and chemical, which are distinctive of the race from which the cell originated; and it is not only that storehouse of characters, but there is present in it a supply of energy-producing materials sufficient to carry it through a shorter or longer period of transformations of form which will result ultimately in a structure able to take in and utilize energy-producing and structure-building materials from its environment. The inactivated egg cell is not to be thought of as a dead thing, but as a living cell in which oxidative processes are taking place at a very slow rate. In most eggs, the series of phenomena necessary to the production of a new individual will never appear unless the egg be fertilized. After a time the egg will die and disintegrate.

Fig. 144.—Cross section of the egg of *Nereis virens* showing spermatozoön entering egg cortex. *sp*, spermatozoön

The process of fertilization involves all of those changes by which two distinct and different forms of germ cells, the ovum and the spermatozoön, are combined into a single cell, the *zygote*. The process of fertilization, the result of which is the activation of the egg, is really a compound of several processes. Activation begins when the sperm cell first comes into contact with the egg substance, and results in a change in the cortex of the egg and in the elevation of the so-called fertilization membrane. This membrane forms with great rapidity, spreading in some cases from the point of contact of the sperm with the egg over the whole surface of the egg within a few seconds of time. The elevation of the fertilization membrane is associated with the giving off of the perivitelline fluid and, in some cases, with a decrease in the diameter of the egg. The perivitelline fluid is hypertonic to sea water and comes to be diluted by water derived both from the egg and from the liquid outside the membrane. The entrance of the spermatozoön into the egg (Fig. 144) is an act which may take place in any one of a variety of ways. In the starfish, there is a drawing in of the sperm head by means of quick streaming movements of the egg cytoplasm which, before the process of fertilization began, had formed long threads reaching out from the surface of the

egg, through the gelatinous envelope which covers it. In other eggs the penetration of the egg by the sperm cell may be slower and may be the result of the activity of the spermatozoön itself. Frequently, streaming movements, of the cytoplasm of the egg, of so marked a character as readily to be seen by the ordinary powers of the microscope, are associated with the processes of fertilization. The egg substance shows itself to be a real dynamic system, the energy supply for which has been previously provided in the yolk material stored within the cell. Also to be included within the processes of fertilization are the remarkable swellings of the colloidal materials of the sperm nucleus, the gradual approach of the egg and sperm nuclei until they come to lie near to each other at some point near the center of the active protoplasm of the egg, the fusion of the germ nuclei into a single nucleus, and, as a final step in the process, the pairing of the homologous chromosomes of the sperm and egg nuclei, so that full capacity for development and inheritance is attained by the zygote.

F. R. Lillie has been able to show that the seemingly simple act of fertilization is really quite an extended series of events in the eggs of Arbacia and of Nereis. Among these events are:

a. Agglutination.—Some substance is given off by the egg which aids in the attachment of the spermatozoön to the egg substance. This is a definite and characteristic reaction, and is reversible.

b. Physical Change.—There is a physical change in the character of the protoplasm of the egg. The egg which was originally in the sol state becomes transformed into a gel. Eggs which were irregular in outline become regular and spherical in form. In the eggs of Nereis, it is noted that the eggs, which before fertilization were spherical in form, become irregular after fertilization, and later assume as spherical form.

c. Sterilization.—When an egg is attacked by a sperm of the same species, a change is brought about which renders it immune to the action of other spermatozoa of the same or of different species.

d. Latent Period.—Following the attachment of the spermatozoön to the egg, there is sometimes a period in which the processes of fertilization appear to be at a standstill. This has been referred to as a latent period.

e. Reaction.– The reaction between the egg and spermatozoön is usually specific, *i.e.*, between germ cells of the same species. It sometimes occurs, under somewhat abnormal conditions, that eggs may be fertilized by the spermatozoa of other species.

f. Penetration.—Fol was the first actually to observe the penetration of the spermatozoön into the egg. He observed that:

> The first spermatozoön which comes into contact with the gelatinous layer surrounding the egg enters it at once, and its point comes into contact with the egg, usually within a second or two. The movements of the tail then slacken,

and the head of the sperm elongates and enters the egg. The tail remains visible for some seconds, then disappears from sight. The head of the sperm forms a small nucleus, "male pronucleus," within the egg. In the starfish he described a small protuberance of clear protoplasm, the *fertilization cone*, arising from the surface of the egg at the point of contact with the spermatozoön, lasting but a few seconds, and appearing to aid in the engulfing of the spermatozoön in the egg.[1]

In the egg of Nereis, the progress of the spermatozoön into the egg is less rapid, and may occupy as much as 50 minutes following the attachment of the spermatozoön to the egg.

g. Membrane Elevation.—Beginning at the point where the spermatozoön comes into contact with the egg, there very quickly arises on the surface of the egg a definite membrane, which separates from the egg and comes to lie at a little distance from the surface. This membrane may continue to exist for some time during the early development of the egg.

h. Increase in Oxidation.—The fertilized egg has been shown to use oxygen at a rate 400 to 600 per cent greater than before fertilization.

i. Increased Permeability of Membrane.—The increased consumption just referred to is associated with an increase in the permeability of the egg membrane to oxygen and to carbon dioxide, and possibly to other substances as well.

j. Decrease in Diameter.—Several observers have noted a definite decrease in the diameter of the fertilized egg, as compared with that of the unfertilized egg. This may be so great as to allow for a diminution of mass to approximately 30 per cent of the original volume.

Effect of Hydrogen-ion Concentration upon Fertilization.—In the discussion, up to the present, there has been the tacit assumption that, whenever the egg and spermatozoön of a given species are brought into close relations, fertilization will take place. The spermatozoa of many animals are inactive so long as they remain within the body of the male, or at least so long as they are in the seminiferous tubules, and only become active when they come into a new environment. The part which the spermatozoön has to play in the process of development makes essential small size and considerable activity. The small size of the sperm cell places a definite limit upon the amount of energy-producing material which can be stored within the cell, and, therefore, upon the amount of activity, *i.e.*, the length of time during which the sperm cell may be active. If the stores of energy-producing materials are exhausted before the cell has an opportunity to play its part in the fertilization of an egg, it is as useless as if it had never been.

Some of the effects of the hydrogen ion upon fertilization have been known since the work of Loeb (1904). Loeb was able to show that normal fertilization does not take place in solutions which are either strongly acid

[1] Quoted from Lillie, "Problems of Fertilization."

or strongly alkaline in reaction. Many students of experimental embryology have found that it is sometimes difficult to secure the fertilization of the eggs with which they were working. This has been especially true of the eggs of the starfish, *Asterias forbesii*, the spermatozoa of which are frequently found to be inactive when they are placed in sea water. If the conditions surrounding the spermatozoa and the egg are favorable, fertilization will take place; otherwise, it will not. All the conditions which may enter into the determination of what is a favorable environment are not known. One of these conditions has been found to be the concentration of the hydrogen ion in the water in which the eggs and spermatozoa are placed. Forms of animals which live in the sea water produce eggs and spermatozoa which commonly interact at the natural hydrogen-ion concentration of the sea water. It happens that occasionally a form is found the spermatozoa of which are inactive at the pH of sea water. Such spermatozoa are ineffective for fertilization. These spermatozoa, however, in many cases may be rendered effective for fertilization by increasing slightly the alkalinity of the sea water.

At the Marine Biological Laboratory at Woods Hole, Mass., the pH of the sea water usually lies between pH 8.0 and pH 8.2. Sometimes, the hydrogen-ion concentration increases so that the pH reaches a value of 7.8, and occasionally it falls below the normal. During the summer of 1925, it varied between pH 8.3 and pH 8.4. The reaction is always on the alkaline side of neutrality. It was found many years ago that, when the spermatozoa are inactive in the sea water, they may be rendered active, and so fit to act in fertilization, by adding to the sea water from 0.2 to 0.6 cubic centimeter N/10 NaOH to 100 cubic centimeters of sea water and spermatozoa.

The range of values of pH, through which normal fertilization of the eggs may take place, is very limited. If the percentage of eggs fertilized in sea water of differing pH values be plotted against the pH values, it will be seen that the curve is an almost symmetrical one (see Fig. 145), rising very rapidly from the zero point, where there is no fertilization, to a maximum at which practically all of the eggs are fertilized, then to drop off suddenly to zero. This curve, plotted from data (possibly insufficient) secured by students in the embryology course at the Marine Biological Laboratory during the summer of 1925, working upon the eggs of *Arbacia punctulata*, illustrates this very characteristic behavior. This curve represents a series of averages, and has been somewhat smoothed in preparation. It is probably true that the curve fails to represent quite accurately the end points of the reaction, and it is possible that further experimentation would modify to some extent the slope of the curve and the breadth of the plateau which marks the optimum pH value for the fertilization process, but the form of the curve may be taken as representing a condition which probably prevails rather generally throughout

the animal kingdom. It indicates that normal fertilization (in Arbacia, at least) takes place only on the alkaline side of neutrality, and has its optimum at approximately pH 8.5. This alkaline reaction serves undoubtedly to increase the rate of metabolic processes of both egg and spermatozoön. This effect is in line with the work of Cohn upon carbon dioxide production by spermatozoa, and also in line with Loeb's results upon the rate of regeneration in the hydroid Tubularia. It should be borne in mind that the determinations mentioned above were made upon sea water as it was circulating through the pipes of the salt-water system at the Marine Biological Laboratory, and the pH values were determined fly the use of indicators. The method was not, therefore, as critical as that used by Clowes and by Smith and Clowes.

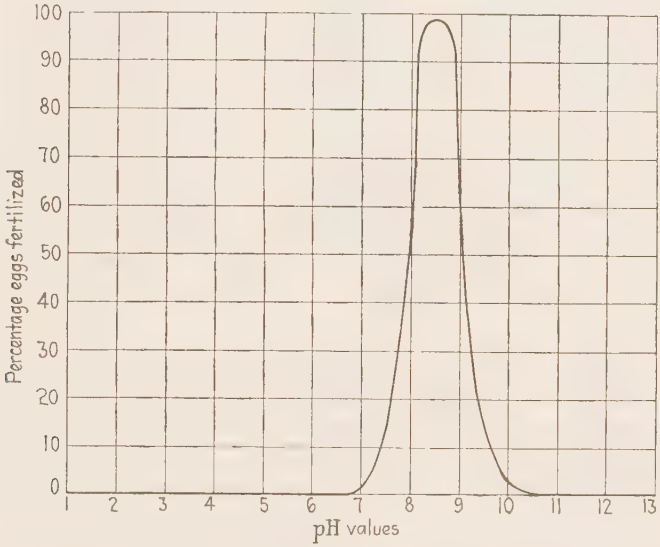


FIG. 145.—Chart showing percentage of eggs of *Arbacia* fertilized in samples of sea water having differing pH values. No attempt was made in these experiments to render the sea water free of carbon dioxide.

Smith and Clowes have determined the ranges of hydrogen-ion concentration, in a few marine forms, through which normal fertilization is possible and in which fertilization is blocked. A typical case is that of Arbacia, and it is all the better suited to our purpose because of results of the class work mentioned in the preceding paragraph. The accompanying curve (Fig. 146), taken from the paper by Smith and Clowes, illustrates the findings of these authors for the form mentioned. Other cases are very similar, though they vary in minute details. These authors found that when the eggs of the forms studied (Arbacia, Asterias, Chætopterus) are inseminated in sea water *free of carbon dioxide* and of varying hydrogen-ion concentration, a block to fertilization appears at a hydrogen-

ion concentration which is constant and apparently characteristic for each species. If the block is defined by the hydrogen-ion concentration in which 50 per cent of the eggs fertilize, these concentrations are: Arbacia, pH 6.8; Asterias, pH 7.0; and Chætopterus, pH 7.1.

Smith and Clowes say:

This block to fertilization is complete, in that eggs either fertilize and develop normally, or do not fertilize at all; and it is perfectly reversible, in that eggs which do not fertilize on the acid side of the block will fertilize immediately if they are returned to solutions on the alkaline side of the block, and inseminated with fresh sperm.

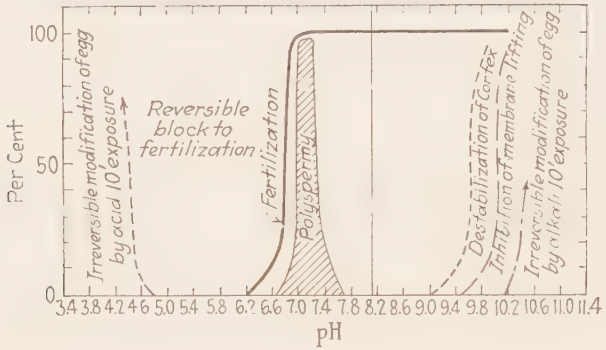

100
50
0
Per Cent
Irreversible modification of egg by acid 10' exposure
Reversible block to fertilization
Fertilization
Polyspermy
Destabilization of cortex
Irreversible modification of egg by alkali 10' exposure
3.4 3.8 4.2 4.6 5.0 5.4 5.8 6.2 6.6 7.0 7.4 7.8 8.2 8.6 9.0 9.4 9.8 10.2 10.6 11.0 11.4
pH

Fig. 146.—The influence of the hydrogen ion on the fertilization of the eggs of *Arbacia*. (*From Smith and Clowes.*)

In sea water more alkaline than pH 9.8 to pH 10.0 the fertilization process in both Arbacia and Asterias eggs is either incomplete or impaired. Apparently, there is no alkaline block to fertilization corresponding in its complete reversibility to the block which appears around neutrality.

In Arbacia there is an increasing incidence of polyspermy within a very narrow range centering at pH 7.2, indicating some critical condition in the mechanism of fertilization at this hydrogen-ion concentration. In Asterias, polyspermy occurs more or less uniformly over a wide range extending from pH 7.2 to pH 9.8.

The form of the curve within whose bounds Smith and Clowes found polyspermy is strikingly like that found for fertilization by the class.

Theories of Fertilization.—It is very natural that different investigators, having attacked the problem of fertilization from different viewpoints, should not agree in their interpretations of the facts observed. Numerous theories as to the nature of the fertilization process have been put forth, and it is not at all to the discredit of the men responsible for these theories to remark that the last word concerning the nature of the process has not yet been said.

Yves Delage bases his ideas of fertilization upon the fundamental concept of the egg as a colloidal system. He holds that the initiation of

development is due to alternate gelation and degelation of the egg protoplasm. There is no question as to the alternation of the sol and gel conditions in the egg, but whether these conditions are to be considered as cause or as effect may be reasonably questioned. It seems likely that there is some process antecedent to the gelation concerning which information is needed.

Heilbrunn regards initiation of development as due to a change in the viscosity of the egg. He finds that the protoplasm of the egg is more fluid before fertilization than after—a finding which other investigators are willing to accept. This change from the more fluid to the less fluid condition is interpreted by Heilbrunn to be the fundamental fact of activation. He says:

> The only physicochemical effect which all parthenogenetic agents possess in common is the production of a gelatinization (or coagulation) within the egg. Hence, I regard this gelatinization (or coagulation) as the direct cause of the initiation of development.

Heilbrunn believes that the so-called fertilization membrane is already existent upon the surface of the egg before fertilization, and that the elevation of the membrane occurs because of the lowering of the surface tension of this membrane. As the surface tension is lowered, the membrane is pushed away from the surface of the egg by forces within the egg which had previously been balanced by the greater tension of the membrane.

Jacques Loeb thought of the egg as a colloidal, physicochemical system which possesses a very highly irritable cortical layer. The fertilization (activation) of the egg involves a cytolysis of the cortex of the egg, induced by a lysin carried to the egg by the spermatozoön. As a result of the action of this lysin upon the cortical layer, a change takes place which, spreading rapidly over the surface of the egg, results in the separation from the surface of the egg of a membrane. This membrane is called the fertilization membrane, and its formation was believed by Loeb to be an indispensable step in the fertilization process. At the same time at which the membrane is elevated from the surface of the egg, other substances leave the egg. The cytolysis initiated by the spermatozoön would spread and lead to the destruction of the egg except for some corrective factor carried into the egg by the spermatozoön. This suggestion of a corrective factor occurs repeatedly in Loeb's writings. In his experimental work upon artificial parthenogenesis, a hypertonic solution was used as the corrective factor. Just has since shown that it is not necessary to postulate such a factor. Loeb contended, also, that fertilization of the egg is associated with a great increase in the rate at which oxidations occur in the egg, an increase of from 400 to 600 per cent being common. Such an increase in oxidation has been confirmed by deter-

minations of the amount of oxygen used by eggs before and after fertilization, and by determinations of heat production before and after fertilization.

F. R. Lillie has made a most careful and extended analysis of the phenomena of fertilization. He finds that the egg is a very irritable physicochemical system. Fertilization is due to the presence of a definite (possibly specific) substance, *fertilizin*. Fertilizin exhibits two kinds of activity: first, it has an agglutinating (or aggregating?) effect upon the sperm; and, second, it has an activating effect upon the egg. Lillie believes that the sperm, through some substance which it bears and which enters into combination with the fertilizin of the egg, thus releases in an active condition this substance within the egg. Dr. Just makes a slight modification in the statement in that he substitutes *aster-forming substance* for *fertilizin* in the preceding sentence. R. S. Lillie has made a critical study of the releasing process mentioned above, and comes to the conclusion that this releasing process cannot be dependent upon the simple acceleration of some chemical process, such as oxidation, for the temperature coefficient of such processes is of the order Q_{10} equals 2 to 3, while in the phenomenon under consideration the Q_{10} equals 200 to 400. He concludes from this fact that:

> . . . there is some change in the nature of degelation or decrease of viscosity in the cortical layer, which presumably allows substances to come together and interact, which in the condition of the cortex of the unfertilized egg are kept apart.

Number of Spermatozoa Required for Fertilization.—It has been commonly assumed by biologists that a single spermatozoön is sufficient to fertilize a single egg. Some years ago this idea was called in question and an attempt was made to show that more than a single sperm cell may in some cases be required to insure fertilization and development. Dr. Lillie has made a reexamination of this matter and has come to the conclusion that in the form upon which he studied a single spermatozoön is sufficient. He based his conclusion upon an experiment involving the theory of probabilities. This experiment involves the use of sperm suspensions of various dilutions. He made use of the eggs and sperm of Arbacia. The sperm with which he began his experiment was the so-called "dry sperm" of the animal, that is, the sperm which exudes normally from the animal, undiluted with sea water. Of this, he made three suspensions:

Sperm 1 = 1 part dry sperm in 100 parts sea water	= 1	per cent sperm
Sperm 2 = 1 part 1 per cent sperm in 100 parts sea water	= 0.01	per cent sperm
Sperm 3 = 1 part 1 per cent sperm in 100 parts sea water	= 0.001	per cent sperm

Following Dr. Lillie's method, the following tests were made by students in the embryology course at the Marine Biological Laboratory:

One drop ($\frac{1}{30}$ cubic centimeter) sperm 1 added to 1000 cubic centimeters sea water containing eggs
One drop ($\frac{1}{30}$ cubic centimeter) sperm 1 added to 3000 cubic centimeters sea water containing eggs
One drop ($\frac{1}{30}$ cubic centimeter) sperm 2 added to 1000 cubic centimeters sea water containing eggs
One drop ($\frac{1}{30}$ cubic centimeter) sperm 2 added to 3000 cubic centimeters sea water containing eggs
One drop ($\frac{1}{30}$ cubic centimeter) sperm 3 added to 1000 cubic centimeters sea water containing eggs
One drop ($\frac{1}{30}$ cubic centimeter) sperm 3 added to 3000 cubic centimeters sea water containing eggs

The concentrations of dry sperm in the different solutions are as follows:

1:3,000,000; 1:9,000,000; 1:300,000,000; 1:900,000,000; 1:3,000,000,000; 1:9,000,000,000.

The curve shown in Fig. 147 indicates the percentage of eggs fertilized in the different solutions as determined by one of the students. In the greater dilutions it is hardly to be conceived that more than one spermatozoön could have entered an egg. It should be stated that the result quoted here has been obtained by others at various times, and is in general agreement with that obtained by Dr. Lillie himself.

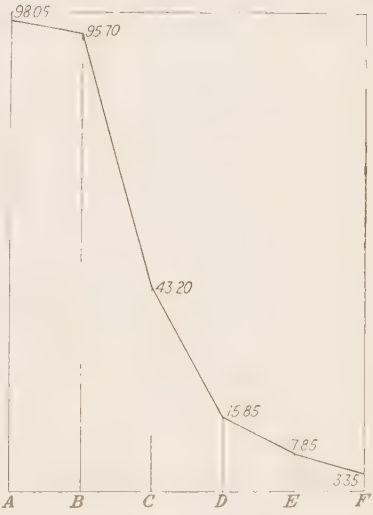


Fig. 147.—Curve showing percentage of eggs of *Arbacia* fertilized in different dilution of dry sperm. Solution A contains 1 part of dry sperm in 3,000,000; B contains 1 part of dry sperm in 9,000,000; C contains 1 part of dry sperm in 300,000,000; D contains 1 part of dry sperm in 900,000,000; E contains 1 part of dry sperm in 3,000,000,000; F contains 1 part of dry sperm in 9,000,000,000.

Heat Production during Fertilization and Cleavage.—The dynamic activity of the egg is immensely increased after the entrance of the spermatozoön. The work involved in the formation or elevation of the fertilization membrane, in the formation of the cleavage nucleus, and in other internal reorganizations of the egg protoplasm causes the expenditure of a considerable amount of energy. The formation of the cleavage spindle must also be costly in energy. All of these processes are associated with, and possibly dependent upon, metabolic processes taking place within the egg. It has been shown by Warburg, and by Loeb and Wastenys, that with the act of fertilization there is an increase in the rate of oxidation of the egg from 400 to 600 per cent above that of the unfertilized egg. This was discovered through making a determination of the rate at which oxygen is removed from the water in which the eggs

are placed. Now, there is also evidence that there is an increase in the rate of heat production of even such small eggs as those of the sea urchin at the time of fertilization corresponding to the increase in oxygen consumption. By the use of very delicate temperature measurements with thermocouples and highly efficient vacuum flasks, it has been determined that the unfertilized eggs of Arbacia give off heat at the rate of 0.08 calorie per hour per million eggs. Immediately after fertilization, the rate of heat production jumps up to about 0.88 calorie per hour per million eggs. This rate of heat production immediately begins to fall, and at about 20 minutes after fertilization comes to a constant rate, which is about 65 per cent of that immediately after fertilization, or 0.57 or 0.58 calorie per hour per million eggs. At the time when the cell passes into the two-cell stage, there is another slight drop in the rate of heat production, to 0.52 calorie per hour per million eggs. Since we know that 1 calorie is equivalent to 4.18×10^7 ergs it is possible to express the heat production value in terms of energy. Thus, at the time of maximum heat production there is an energy expenditure of about 37 ergs per egg per hour. After a few minutes this is reduced to about 24 ergs per egg per hour, and at the passing of the egg into the two cell stage to 21.8 ergs per egg per hour. The great evolution of heat immediately following the contact of egg and sperm is very likely associated with the elevation of the fertilization membrane.

Artificial Parthenogenesis.—It is doubtful if any single line of physiological investigation has attracted so much attention from scientists and the general reading public as that of artificial parthenogenesis. The suggestion that it might be possible, by artificial means, to induce the development of an egg cell, seemed to some to be a long step toward the solution of the riddle of life. It is not possible here to give a history of the steps in working out the methods of the artificial initiation of development, nor in the evolution of the modern conceptions of the process of fertilization. The names of many investigators, American and European, simply give a hint as to the intense interest involved in the work, and of the men who have contributed to our present information concerning the matter. Among these may be mentioned Morgan, Wilson, Loeb, Delage, Mathews, Fischer, Heilbrunn, McClendon, Garrey, Greene, Lillie, Meade, Moore, and Just. The list, of necessity, must be incomplete.

Hertwig had noted that certain toxic substances would cause the eggs of Echinoderms to divide, even though spermatozoa were not present. Some similar observations had been made by Morgan and one of his students. It was not until Loeb saw the possible significance of the facts that interest in this line of work became intense. Incidentally, it was the brilliancy of Loeb's genius that has added much to our knowledge

of the facts of fertilization and has served as a wonderful stimulus to other investigators.

Students of development had noted that normal fertilization results in two distinct effects upon the egg—it initiates cell division, and it also provides for biparental inheritance. Loeb tried to solve the mystery of fertilization by an attempt to imitate the process. Others have attempted to make an actual analysis of the phenomena of fertilization. The latter is, of course, an exceedingly difficult thing to do, as the processes are very complicated. When it was found that it was at all possible to induce development by artificial means, there were many attempts on the part of a large number of investigators to discover what might be the fundamental principle involved in fertilization. It is not to be wondered at that a great variety of methods of experimentation were employed, or that for a time the results of the work seemed to be contradictory. Here, again, the fact was brought out that it is sometimes difficult to distinguish between facts which are of fundamental importance and those which are merely incidental. It was recalled that the process of normal fertilization results in a change in the cortex of the egg of such a nature that a membrane is lifted from the surface of the egg. The presence of this membrane had been taken simply as a convenient indicator of the fact that fertilization had taken place. It now appeared that the formation of the membrane is a matter of fundamental importance, and that almost any method which will induce the formation of the membrane will induce cell division. The formation of the membrane is, however, caused by some agent which induces a superficial cytolysis of the egg substance, so Loeb concluded that any method which will induce this superficial or cortical cytolysis, without endangering the rest of the egg, may be considered as a parthenogenetic agent. Among the substances which will induce such cortical changes in the eggs of Echinoderms may be mentioned such substances as saponin, the bile salts; the lipoid solvents, benzene, toluol, amylene, chloroform, ether, alcohols, etc.; bases, hypertonic and hypotonic solutions; rise of temperature, lowering of temperature; the chlorides of barium and strontium; blood extracts of foreign species, but not of the same species; extracts of the sperm of foreign species, but not of the same species; in the starfish, mechanical agitation; and in amphibian eggs, simply pricking with a sterile needle.

Loeb found that, if the eggs of Arbacia were placed in a mixture of 50 cubic centimeters sea water plus 2.0 cubic centimeters N/10 butyric acid for from 2 to 4 minutes, then transferred to sea water for 10 to 15 minutes, then to 50 cubic centimeters sea water plus 8.0 cubic centimeters $\frac{5}{2}$M NaCl for from 17½ and to 22½ minutes, and then washed in normal sea water, the eggs would segment, live, and develop into normal plutei. In this case the treatment with the butyric acid was held to be responsible for the superficial cytolysis of the egg and the formation of

the fertilization membrane. The action of the acid was such as to lead to too great a cytolysis, unless the treatment with the butyric acid was followed with some corrective agent. Such was the treatment with the hypertonic solution.

Dr. Just has recently presented evidence which throws some doubt upon the validity of Dr. Loeb's interpretation of the facts observed. He has shown that treatment with hypertonic sea water (Loeb's anticytolytic corrective factor) alone may serve as the parthenogenetic agent with the eggs of Arbacia and Echinarachnius. Other forms, such as the starfish, etc., need no such corrective factor. Hypertonic sea water, made by adding 20, 22, or 24 parts of $\frac{5}{2}$M NaCl or KCl to 80, 78, or 76 parts of sea water respectively, has the effect upon Arbacia eggs of causing the elevation of the so-called fertilization membranes while the eggs are still in the solutions. The time for the reaction varies with the strength of the solution, 5 to 10 minutes being required for the solution containing the proportions 24:76 of NaCl solution and sea water. The membranes produced by the eggs during this treatment separate from the eggs somewhat more slowly than the membranes formed by natural fertilization, but they are as clear and as distinct and possess as wide a perivitelline space as normally fertilized eggs.

As in normal fertilization, there is here also a decrease in the diameter of the eggs due in part to the passing of water out of the eggs, and also to the giving off from the egg cortex of substances which, going into solution in the water contained in the perivitelline space, give to that liquid a higher concentration than exists in the sea water. This membrane, being more permeable to water than to the dissolved substances, becomes very tense and stretches somewhat. At a later period the osmotic pressure, inside and outside the membrane, becomes equalized, probably through the diffusion of salts outward, and the membrane becomes less turgid. Finally, by the time of the blastula stage, the membrane is lost.

Dr. Just emphasizes the fundamental importance of the change which takes place in the egg cortex, and which he refers to as the cortical reaction. He holds that the fertilizability of the egg depends (in some cases) upon the presence in the cortex of the egg of a substance known as fertilizin, as had been shown by Lillie. Complete fertilization of the egg depends upon combination of the fertilizin with the sperm. The cortical explosions leading to the separation of the membrane are the sign of the complete fertilization reaction.

The artificial initiation of development has been brought about by a variety of means, and in many forms. Among the animals in which satisfactory results have been obtained may be mentioned Arbacia, Strongylocentrotus, Asterias and other Echinoderms, Amphitrite, Thalassema, Mactra, Polynoe, Nereis, Ophelia, Chætopterus, Petromyzon, Rana, etc. To bring about these results, treatment with carbon dioxide,

bile salts, lipoid solvents, bases, fatty acids, hypertonic or hypotonic solutions, heat, cold, mechanical agitation, treatment with the body fluids of foreign species, cell extracts of foreign species, sperm extracts of foreign species, etc. have been employed. It can be readily seen that to find the common factor in all of these different methods of treatment has not been a simple matter.

Parthenogenetic Development in Nature.—It has long been known that the unfertilized eggs of many animals develop into normal individuals. Among bees, ants, and wasps, the unfertilized eggs develop into males. Among some of the fresh-water Crustacea, males either do not occur at all or only very rarely.

It has been stated that the meaning of fertilization is the restoration of the lost power of nuclear and cell division. During the period when the germ cells are growing in the ovary, they come to a point where it is impossible for them to divide further. The process of fertilization restores this power. It has been also stated that the eggs of some animals —for example, some of the Echinoderms of the eastern Atlantic coasts— occupy a somewhat transitional position between forms which are naturally parthenogenetic and those which are not. At any rate, the eggs of some of these forms, and even of the starfish, *Asterias*, of the western Atlantic, are so delicately adjusted that only a slight mechanical disturbance is necessary to initiate cell division. The suggestion has been made that some of these forms, whose eggs may be caused so readily to segment and develop, should be included among the naturally parthenogenetic forms.

Physiological Evasions of the Hazards of Development.—Among the lower metazoa, there is a tremendous waste of valuable reproductive material because the developing eggs are scattered far and wide and meet with a great variety of conditions, some of which lie so far to one side or the other of the optimum that continuance of development is impossible. In addition to the ordinary hazards due to variations of the environing medium, the attacks of enemies, who find the rich protoplasm of the eggs and embryos a most desirable supply of food must be taken into account. So, with all the vicissitudes through which the eggs must pass because of enemies and unfavorable circumstance, it happens that the total number of adult individuals of any given species does not rapidly increase, although a single adult female may produce thousands or sometimes millions of eggs in a single season.

In order to obviate the tremendous waste of energy and material, a variety of methods of conservation have been devised in the various animal groups, and, as the methods of conservation have become more and more adequate, the amount of reproductive material produced by a given female has been correspondingly reduced. Also, as the methods of conservation have been improved, there has been an associated increase

in the complexity of the organism developed; so it may be said that complexity of adult development of any given species is in a way dependent upon the degree to which the conservation of reproductive material has been carried.

It is impossible in such an account as this to go into any extended discussion of the various forms of conservation. Among the methods employed to make more certain the viability of the developing individuals, the following may be mentioned:

Egg laying in masses of various forms: strings, balls (Mollusca, Amphibia).

Nest building (fishes, Insecta, Reptilia, Aves).

Egg attachment to solid object, singly or in groups: capsule formation (Amphibia, Mollusca, some fishes, Crustacea).

Egg carrying by adult, attached to surface of body, to appendages, in skin folds, pockets, etc. (Crustacea, Amphibia).

Increased storage of yolk, provided for prolonged development (Mollusca, Crustacea, Reptilia, Aves).

Albumen coat and shell, as protection (Reptilia, Aves).

Internal development, eggs provided with small amount of yolk, nutrition derived from mother (ovovivaparous, as in sharks, dogfishes, etc.; viviparous as in mammals, showing gradations from Marsupials up).

It is to be understood, of course, that this exhibition of care for developing offspring is not at all an abrupt development, but a very gradual evolution. Even among the mammals there is a very marked evolution in the development of the process from the egg-laying forms such as the Echidna, through the Marsupials, up to the Primates. In the higher phyla there is not only the protection against accidents so fatal to a very high percentage of the fertilized eggs of lower forms, but there appears to be a definite attempt to provide for the embryo an environment which shall, in its main features, very closely approach what may be considered to be the optimum conditions for the development of the species in question, and which is, in effect, a very close approach to the conditions of the original aquatic environment in which the ancestors of the species passed their developmental stages. Certainly, in the mammal, there is provided a constancy of temperature, oxygen, salt balance, osmotic pressure, hydrogen-ion, and other conditions, which is unequaled in the developmental condition of any other form.

Following fertilization, the egg undergoes a series of rearrangements which finally terminate in cleavage. In some eggs, as in that of the frog or some of the Ascidians, these rearrangements are so far-reaching as readily to be observed. In other eggs these rearrangements may be wholly invisible—yet, it seems certain they must occur. In many eggs there is little optical evidence that the fertilized egg is different from the unfertilized egg. If unfertilized eggs and those which have just been

fertilized are centrifuged a great difference in their physical properties will be seen. The unfertilized egg has its material thrown into definite zones in such a way as to indicate that the egg is composed of several different kinds of substance. The fertilized egg is much more resistant and does not so readily take on a zoned appearance. In the unfertilized egg the protoplasm appears to be in a more or less liquid condition. The fertilized egg appears suddenly to have jelled, and so resists the movement of the visible particles held within the cytoplasm. Notwithstanding this gel condition, there are movements of substance within the egg. Materials which previously had been diffusely scattered throughout the cell are now collected into definite localities within the egg. This has been beautifully shown, by Conklin, to be true with the eggs of Ascidians, and by Morgan in the case of the frog.

Types of Cleavage.—Students of embryology are accustomed to classify the ways in which the cells resulting from the cleavage of the egg arrange themselves as:

Total, or holoblastic:
- Radial
- Bilateral
- Spiral

Partial, of meroblastic:
- Superficial
- Discoidal

There can be no question that the amount of food yolk in the egg in proportion to active protoplasm and the distribution of the food yolk in the egg are factors which have an important rôle in the determination of the cleavage type. Other factors also exist, but they are less evident to the casual observer. Observations which have been made upon the cleavage of different eggs have led to certain conclusions which may be summarized in the following generalizations:

1. Sach's rule: Successive divisions of the egg tend to take place at right angles to each other.

2. Balfour's rule: Yolk matter impedes division.

3. Hertwig's rule: The nucleus of the cell tends to occupy the center of the cytoplasm and the dividing nucleus, or spindle, elongates in the direction of the greatest protoplasmic mass, or (Pflüger) the direction of least resistance.

Development a Series of Interactions.—It has been stated that the normal development of an egg involves a series of interactions between the egg and its environment. The physicochemical system, which is called the egg, is not sufficient of itself to produce a normal embryo. This must mean that, even before fertilization and cleavage, the egg is taking something from the environment, and giving something to it. It is known that the egg carries on a respiratory process in which it takes

in oxygen and in which it gives off carbon dioxide; that it gives off heat. It is known that older embryos and adult organisms give off nitrogenous wastes. The living egg must do the same, though it must be confessed that this has not been experimentally proved. The food which the egg needs has been stored up in it during a long period of growth, and this is available in a form easily acted upon by the cellular enzymes, and then absorbed.

What is the nature of this environment? For the eggs of most of the marine animals, it is the sea water; for the eggs of insects, it may be the earth, decaying vegetation, fresh water, etc.; for the eggs of the amphibia, it is fresh water; for the eggs of reptiles and birds, there is included within the shell of the egg an environment which takes the place of the original sea; for the eggs of the mammals, the environment is the liquid secreted by the tissues of the mother.

Now, while these different forms of environment seem to be very different, they have some things in common. They provide for the egg an environment which will supply the necessary amount of oxygen, and carry away the accumulation of carbon dioxide which results from the oxidative processes going on within the cells. They provide environments having certain inorganic salts present in certain definite proportions (which are very similar for all forms). They provide environments having total concentrations of salts such as those to which the tissues of the adults of the same species are accustomed. In other words, the environment in each case is provided by a solution having a content of certain molecules, certain positive and negative ions, in a definite concentration, thus giving a definite osmotic pressure, a definite hydrogen-ion concentration, and a definite (within limits) temperature. Through long periods of ancestral life, the protoplasm of each species of animal has become so related to its own particular physicochemical environment that any great deviation from the normal may lead to modification of development, or disaster.

It is very probably true that the eggs and developing embryos of different species are not equally susceptible to environmental changes, and that the eggs of a given species are not all equally susceptible at all stages in their developmental careers. Definite modifications in the environmental conditions occurring at special times, when certain important processes are taking place, are likely to have a greater influence than if the same environmental modifications were to occur at other periods. Stockard has referred to these times of high susceptibility as "critical periods." At such times changes of temperature, of osmotic pressure, or of chemical composition of the environing liquid are much more effective, and much more dangerous to the developing individual than they are at other periods. Investigation has not yet revealed just when, in the course of development, these periods occur. The field of experi-

mental embryology is still open for much investigation and for real discovery.

Effects of External Factors on Development.—It is not possible here to review all of the work which has been done in experimental embryology. It seems essential, however, to point out a few facts that indicate something of the nature of developmental processes, and the necessity of maintaining the proper relations between the developing egg and its environment.

1. *The Effects of Pressure.*—The effects of pressure have been studied in relatively few forms, and in these the pressure has been allowed to act through only a brief portion of the embryonic history. Driesch found for the eggs of the sea urchin, and Wilson for the eggs of Nereis, that the blastomeres could be forced to arrange themselves in an unusual manner and still give rise to a perfectly normal embryo. Instead of forming a normal blastula, the cells may be forced to arrange themselves in the form of a plate, one cell in thickness. This is due to the fact that the external pressure forces the eggs and the cleavage cells to lie with their major axes parallel, or nearly parallel, to the pressure planes, the space between these planes being too narrow to allow them to assume their usual positions. If the pressure were long continued, of course, it would be impossible for the eggs to produce viable embryos. If, however, the pressure be removed at an early date, the blastomeres will rearrange themselves and give rise to normal embryos. This may occur even though the pattern of cleavage, as well as the arrangement of cells, has been disarranged. In these experiments it is necessary to maintain a constant supply of oxygen. Unless this is done, the development of the eggs will be abnormal or cease.

2. *Influence of Osmotic Pressure on Development.*—If fertilized eggs of marine animals, such as the starfish or sea urchin are placed in hypertonic solutions or hypertonic sea water (*i.e.*, sea water which has been concentrated by evaporation or by the addition of salt), it is found that water is given off from the egg, and the egg decreases somewhat in diameter. If, for example, 2 grams of NaCl are added to 100 cubic centimeters of sea water and the recently fertilized eggs of Arbacia are placed therein, it will be found that the cytoplasmic division is inhibited, although the nuclear division may still occur. After a time, numerous asters may be found in the cytoplasm, indicating that nuclear activities have not been inhibited, though they may have taken place more slowly than is normal. A still more concentrated solution will have the effect of completely inhibiting the division of the nucleus. If, after numerous asters have been formed in the cytoplasm, the eggs are replaced in the normal sea water, they will be found quickly to break up into as many cells as there were asters formed. Such eggs will continue to develop and will form normal embryos.

Among animals whose eggs develop in fresh water, or whose eggs are provided with a hard shell, or which retain the eggs within the body during development, there is not likely to be any interference with the ordinary processes of development from a reduction of the osmotic pressure of the surrounding medium. With marine animals, however, there is always the possibility that the osmotic pressure of the surrounding medium may be reduced. If such reduction of the osmotic pressure of the surrounding medium does take place, the behavior of the eggs will depend somewhat upon the degree to which the reduction occurs. If it is very slight, there may be no visible effect upon the eggs; if it is greater, a separation of the blastomeres may result, as is found with eggs caused to develop in calcium-free sea water. If there is a marked reduction of the osmotic pressure of the sea water soon after the fertilization of the eggs, there frequently follows an endosmosis of water which results in the rupture of the fertilization membrane, so that a portion of the egg substance flows out and forms what is known as an exovate. When the first cleavage takes place, one of the daughter nuclei frequently migrates into this exovate, presumably because the addition of the exovate to the original form of the egg determines the greater diameter of the egg and, therefore, the position of the first cleavage spindle. Each part of the egg then continues to divide and to attempt to form a blastula. The success of this attempt will, in part, be determined by the width of the neck connecting the two portions of the egg. If the neck is narrow, two blastulæ will be formed; if it is wide, only a single blastula will appear. If two blastulæ are formed, each will attempt to form an embryo. In the various stages falling between the formation of two distinct and separate blastulæ and only one, there arises the opportunity for all sorts of intermediate conditions in which there is an apparent fusion of parts of two individuals. Such individuals are referred to as Siamese twins.

3. *Effects of Temperature upon Development.*—There are two ways in which temperature may affect living cells. The first is by a sudden change from one temperature to another over a considerable interval, and yet not subjecting the cells to temperatures which would be necessarily injurious. Such a sudden change may result in what may be thought of as a shock effect, a stimulation of the protoplasm. If, for example, the unfertilized eggs of the starfish are subjected to a sudden change of a number of degrees, either above or below the normal temperature of the sea water, and then the eggs are brought back into the normal temperature again after a few seconds or a few minutes, it will be found that a certain proportion of the eggs will form membranes and will segment as if they had been fertilized. The exact duration of exposure and the interval through which the temperature change must be made, to bring about this result, are matters of experiment and must be determined for each lot of eggs used. In general, it may be stated that the greater

the temperature change the shorter the period of exposure necessary to bring about cleavage. For example, if eggs are taken from sea water at a temperature of 21° and placed in sea water of 35° for 70 seconds and then brought back to 21°, a certain proportion of them will segment. If they are raised to 38°, the time must be reduced to about 20 seconds to effect the same result. Similarly, eggs may be placed in water at reduced temperature. If, after maturation, the eggs are transferred to water of 3°, it will be found that they must remain at this temperature for 2½ hours before being brought back to normal temperature in order to effect the same result as was obtained with heating. There is probably in the exposure to cold a combination of the stimulating effect of the wide shift in temperature and the slowing up of chemical processes in the cell due to the lowered temperature.

The second effect of changes of temperature upon developing eggs which should be noted, is the modifying effect upon metabolic processes. This appears to be just as true and just as significant in eggs as in adult animals. The rate of cleavage is markedly affected by changes of temperature; and the temperature coefficient is of such a magnitude as would be expected, if cell division is primarily dependent upon some chemical reaction. The following figures, taken from Loeb's "The Organism as a Whole," give an idea of the time required for the fertilized egg to pass into the two-cell stage at different temperatures.

TABLE 55.—EFFECT OF TEMPERATURE UPON THE TIME REQUIRED FROM INSEMINATION TO THE FIRST CELL DIVISION

Temperature in degrees Centigrade	Arbacia		Strongylocentrotus
	Loeb and Wastenys 1911	Loeb and Chamberlin 1915	
3			532
4			469
5			352
6			275
7	498	.....	291
8	410	411	210
9	308	297.5	159
10	217	208	143
11	175	175	
12	147	148	131
13	....	129	
14	....	116	121
15	100	100	100
16	85.5		
17	70.5		
18	68	68	87
19	.....	65	78
20	56	56	75
21	.....	53.3	78
22	47	46	75
23	.....	45.5	Upper temperature
24	.....	42	limit
25	40	39.5	
26	33.5		
27	34		
28			
29			
30	33		
31	37		

TABLE 56.—TEMPERATURE COEFFICIENTS CALCULATED FROM ABOVE DATA

Temperature range	Arbacia Q_{10}	Strongylocentrotus Q_{10}
3 –13	...	3.91
7 –17	7.3	3.27
9 –19	4.7	2.04
12 –22	3.1	1.74
15 –25	2.5	
17.5–27.5	2.2	
20 –30	1.7	

It already has been pointed out that different chemical processes are differently affected by similar changes in temperature; *i.e.*, all do not

have the same temperature coefficients, nor do all have the same optimum points. This is just as true of chemical operations which take place within eggs as elsewhere. A glance at Tables 55 and 56 will indicate the fact that, while the cleavage processes of the two forms Arbacia and Strongylocentrotus undoubtedly have chemical reactions at their bases, they are quite different processes, or there is a different balance of chemical processes which are going on at the same time. These different chemical reactions of the egg are differently influenced by the changes of temperature. Some are accelerated to a greater amount than others by a 10° rise in temperature. Some, possibly, are actually diminished by a rise in temperature after a certain point is passed. This means that the balance of reactions within the egg is upset. Some substances are being produced at a faster rate than they ought to be, in proportion to the rate of production of other substances. The result is that the chemical work of the cells suffers an interference which results in abnormality. The temperature coefficients in these two cases seem to indicate that, at the temperatures which are normal for the animals concerned, the reactions which underlie cell division are chemical in character. They also indicate that, as the temperature is raised considerably above that which is normal for the animal, there is an interference with the chemical balance, possibly through the slow destruction of some cell catalyst, so that the increase in the rate of cleavage is much lessened.

It should be further noted that the temperature changes need not be extreme to bring about very marked changes in the metabolic work of the cells. To bring the eggs of the sea urchin into an environment only a few degrees above the normal has been found to result in a peculiar type of abnormal development. Driesch has shown that a somewhat elevated temperature is responsible, with the eggs of Strongylocentrotus, for the formation of embryos in which the endoderm evaginates during the formation of the gut instead of invaginating in the normal way. A similar observation has been made by the present author upon the eggs of Arbacia which were allowed to develop under conditions of somewhat elevated temperature. In the latter case, the exact temperature was not noted, though it was not much in excess of the normal. The eggs were developing upon the water table of the Marine Laboratory during a period of warm weather.

Kellicott has called attention to the fact that his observations upon the development of the embryos of the minnow Fundulus bear out Loeb's statement that "the low temperature only retards the chemical reactions underlying development" to a certain point. Loeb had stated a temperature of 0 to 2° would inhibit, or greatly retard, embryonic growth, but that, when the eggs were again brought into a normal temperature, they would develop normally, and would produce normal embryos. Kellicott found, with slightly higher temperatures than Loeb

mentioned, that a very large percentage of the eggs developed abnormally and produced monsters of various sorts. Lillie and Knowlton had previously found that the eggs of the frog, *Rana virescens*, develop abnormally, if at all, at 2 to 3°C. Other influences than that of temperature are also known to produce comparable results. Mall has studied the factors which influence the production of monsters among vertebrates and concluded that: "A monster is due to the influence of external substances which retard the growth of the embryo, usually in one portion more than in another." Mall believed that some parts of the embryo are more susceptible to experimental insults than others, and that the total effect of the abnormal condition is to be explained upon the supposition of a faulty nutrition of the embryo during development. Kellicott believed that:

> The causes underlying the abnormal development are to be sought in some derangement of the developmental mechanism or relations of the diverse components of the very early stages of the organism.

Kellicott found in his experimental material that there was much visible modification in the appearance of the eggs subjected to unusual temperatures. While there were many cell nuclei which appeared to be normal, there were irregular, large and small, masses and fragments of nuclear material scattered about through the cytoplasmic portions of the eggs – and frequently there were no cell walls corresponding to these nuclear masses. The protoplasm had taken on the form of a syncytium. This is possibly an effect which is comparable with the syncytial condition found in eggs which have been treated with a slightly hypertonic solution. He found also:

> . . . masses of granular cytoplasm which somewhat resemble the material of the uncleaved germ disc, and masses of clearer, possibly protoplasmic, substance usually in the form of vacuoles which slightly resembled that mass of clear protoplasm which in normal development forms, for a brief period preceding cleavage, a lens-shaped disc on the lower side of the germ disc.

Kellicott suggested that the cause of the abnormal development is to be found in a disturbance of the normal organization of the ovum, as expressed by the unusual characters and distributions of the differentiated materials of the egg protoplasm. It occurs to the present author that it may be possible to associate this modification of the distribution of cellular materials with the various physical and chemical processes of egg metabolism whose balance is disturbed by the marked change of temperature.

4. *Isolation of Blastomeres.*—Attention already has been called to the fact that protoplasmic cells behave in accordance with the same laws that apply in the formation of systems of non-fusing drops of inorganic materials; they maintain their separate unfused condition only if there

are between them walls of material which is insoluble in the substance of the drops or in the surrounding medium.

In some eggs, it is not easy to observe any such membranes surrounding the cells; in other cases they may very readily be seen. In the eggs of the starfish, *Asterias vulgaris*, there appears to be, in the early-cleavage stages, a very complete separation of the blastomeres from each other. Sometimes very narrow protoplasmic bridges are to be seen connecting one cell with another. In these eggs the limiting factor which apparently determines the distance by which the individual blastomeres may separate from each other appears to be the fertilization membrane. In other eggs there appears, as in Arbacia, to be a thin film of material lying around and between the blastomeres, and which apparently serves to hold them together. This material is not soluble in sea water. In some of the Echinoderms, it was found possible by Herbst to reduce the coherence of the cells for each other by placing them in sea water lacking in calcium. In these eggs the films dissolve and the blastomeres separate. A study of the development of such isolated blastomeres from the eggs of different animal groups has led to a general conclusion which has been stated by Przibram as follows:

> In different zones of the egg, different (chemical) substances are present which normally cause the differentiation of the various organs in their proper regions. If these substances become distributed elsewhere by cleavage, gastrulation, and the later processes of differential growth they produce the same effect in their new positions. If a reduction of the egg material, unaccompanied by a rearrangement of the various substances, is made, the prospective significance of the blastomeres leads to a self-differentiation of the reduced portions, whereby one-half, one-quarter, and one-eighth . . . forms arise; if, on the other hand, a rearrangement of the egg content is possible in such a way that all the substances occupy the same positions with regard to each other as in the intact egg, perfect forms arise on a proportionally diminished scale; the prospective potency of such blastomeres is greater than their prospective significance.

In some forms of animals, the separation of these *organ-forming substances* and their distribution to definite blastomeres occurs at an earlier date than in other animals. In the eggs of some of the Annelida, Mollusca, Ctenophora, Tunicata, the separation takes place at an early date in the cleavage of the egg, if, indeed, it has not occurred before cleavage takes place at all. On the other hand, there is evidence that, in some of the Hydrozoa and in the Echinodermata, this separation and distribution may take place at a later date. It may be well to state here that recent experiments have thrown some doubt upon the earlier statements of Driesch and others to the effect that from a single blastomere of the egg of the sea urchin, in the eight-cell stage, a complete and normal embryo may arise.

5. *Effect of Variations in the Chemical Environment.*—The egg must be thought of as a very heterogeneous system undergoing a series of changes under the influence of an external medium. For all marine forms the sea water serves as this medium. In animals living in fresh water, or which deposit the eggs in water, it serves as the medium. In animals which retain the eggs within the body during development, the body fluids serve as the surrounding medium. These different environing fluids appear to have suffered little modification, even during quite extended periods of racial history. Each species of animal has come, therefore, to produce eggs which have the power to develop normally in its own particular type of medium. It must not be thought that there is here any conscious adaptation on the part of the animal to the environmental conditions. It may be that, in embryonic development, only those eggs have lived which have been in close accord with their environment, and other have perished. Whatever the cause, the result has been that there is a very close coordination between the egg and its environment.

The effects of changes of the total concentration of substances present in the sea water have already been considered. It is now necessary to consider the effects of variations in the relative proportions of the chemical constituents of the environing liquid. For this purpose, the sea water will be used as a type. The sea water is an exceedingly complex solution, containing about 3.5 per cent of various salts in solution. Between 85 per cent and 90 per cent of these salts are ionized. The water itself is ionized to a slight extent (10^{-14}). It is, therefore, a solution of molecules and ions in water. Of these ions there are: four electropositive (kations): Na', K', Mg'', Ca'', and four electronegative (anions): Cl', Br', SO_4'', CO_3'', which are most abundant, and by most workers considered to be most important. There are also traces of N, Ph, I, Fe, Mn, Ag, etc., etc. It can be readily understood that, in such a mixture as this, there are almost incalculable possibilities of chemical combination. A few of these possibilities are indicated in Fig. 17, (p. 152). Inasmuch as there are many other molecules and ions present in solution, the number of possible combinations becomes immensely increased. The modifying effects of local conditions in restricted areas, such as the inflow of large amounts of fresh water from large rivers, the addition of large amounts of sewage with its bacterial life, variations in the oxygen supply due to the presence of large numbers of bacteria, or to the presence of great quantities of green algæ, variations in the carbon dioxide content of the air, etc., can well be imagined to have important effects upon the composition of the sea water as regards the embryonic development of many aquatic forms. Variations in the carbon dioxide content of the water would certainly lead to variations in the hydrogen-ion concentration, and might well come to be a determining factor in the life of many a marine organism.

McClendon has shown that an artificial sea water may be synthesized as shown in Table 21 (p. 154). This solution, exposed to the air until it takes on the pH of the normal sea water, is a very close approximation to normal sea water, having the same salts in essentially the same concentration. It is, therefore, to be considered as a balanced solution in the same sense as the blood or the lymph is to be so considered.

Since sea water is so complex it can be readily understood that it may easily become somewhat modified in its composition. These modifications may have the effect of changing the relative amount of oxygen, carbon dioxide, sodium, potassium, magnesium, calcium, sulphate, etc., present at any time. It has been a matter of interest to experimental workers to discover, if possible, whether each or any of these constituents of the sea water may be considered to have specific effects. To do this it has been necessary to work with artificial solutions. Many of the early experiments were made by the use of Herbst's formula for sea water:

Table 57

$NaCl$	3.0 per cent
KCl	0.07 per cent
$MgCl_2$	0.32 per cent
$MgSO_4$	0.26 per cent
$CaSO_4$	0.1 per cent
$CaHPO_4$	in small quantities
$Ca_3P_2O_8$	in small quantities
$CaCO_3$	in small quantities
Fe_2CO_3	in small quantities
Si	in small quantities
Br	in small quantities
I	in small quantities

Later experiments have been made by the use of Van't Hoff's formula

Table 58

100 cubic centimeters	$M/2$ $NaCl$
7.8 cubic centimeters	M 2 $MgCl_2$
3.8 cubic centimeters	$M/2$ $MgSO_4$
2.2 cubic centimeters	$M/2$ KCl
1.5–2.0 cubic centimeters	$M/2$ $CaCl_2$
Trace	$M/2$ $NaHCO_3$

This solution has proved to be a very useful one, partly because of the ease with which it can be handled. It is to be noted that it does not contain all the substances found either in McClendon's artificial sea water or in Herbst's solution. It was thought that a solution containing those substances which are present in the largest amounts, in approximately the concentrations in which they exist in the sea water, would serve the

experimental needs. In general, this is probably true. For long-continued experiments, however, it would seem to be wise to have the artificial solution as nearly like the actual sea water as possible, with the exception of the single ion whose effect is under investigation. It may be that some of the substances which are found in the sea water in very small concentrations may yet be very important. If this shall prove to be true it may be necessary to revise some of the findings of experimental embryologists because of the omission of these substances.

It is also possible that some of the effects which have been noted as specific effects of the various substances may prove not to be such, but rather be due to an interference with development at some particular phase of the process when the egg was more than normally sensitive to change in its environment, and which might have been induced by some other factor as well as by the one employed.

6. *Effects of Oxygen.*—The sea water normally contains oxygen in solution. The amount of oxygen which may be found in any given sample of sea water is subject to great variation because of local conditions. Such variations may be of a degree seriously to affect the normal respiratory processes of egg cells. Even before fertilization the egg is undergoing a slow oxidation. This rate of oxidation is greatly increased by normal fertilization or by the membrane-elevating processes of artificial parthenogenesis. Following fertilization, there is a continuous demand upon the part of the egg or the growing embryo for a supply of oxygen. As the size and the activity of the embryo increases, the demand becomes more intense. The gradual solution of the egg membranes of unfertilized eggs in the water and the ensuing cytolysis of the eggs under conditions of insufficient oxygen supply are matters of everyday experience to workers in the field of experimental embryology. Such cytolysis may be retarded and the life of the eggs prolonged by submitting them to the action of very dilute solutions of potassium cyanide or to the action of hypertonic sea water.

It is not alone the eggs of the lower forms which are affected by variations in the oxygen supply. The eggs of the fish Ctenolabrus are what are known as pelagic eggs. They normally float at the surface of the water and so receive a generous supply of oxygen. If they are subjected to a reduced supply of oxygen, they behave somewhat as do the eggs of Arbacia in a hypertonic solution: They form many asters, and, upon being restored to normal sea water with its normal oxygen supply, they may divide into as many cells are there are asters present. The cell membranes apparently liquefy in the absence of oxygen. The effect is somewhat like the effect of the lack of calcium in so far as the membranes are concerned. Segmentation ordinarily does not go beyond the eight-cell stage. It is interesting to note in this connection that exposure to an excess of carbon dioxide is quickly fatal to these eggs.

The minnow Fundulus produces eggs which normally lie on the bottom (demersal eggs), and have a somewhat greater resistance to a reduced oxygen supply than have the eggs of Ctenolabrus. If they are exposed after fertilization to an atmosphere of hydrogen, they will continue to segment for a period of 24 hours, but do not have the capacity to form embryos. The capacity of the eggs of Fundulus to endure lack of oxygen decreases with development. Eggs which are just fertilized may continue for 4 days in lack of oxygen, and at the end of that time, if returned to a normal environment, will continue to develop. This time is reduced to 24 hours for embryos which have developed to the point at which circulation is established before they are subjected to the adverse condition. Carbon dioxide is quickly fatal.

The eggs of the frog develop in the shallow water of fresh-water ponds. If the oxygen supply is reduced by as much as one-fourth of the atmospheric pressure, there is a high mortality of the eggs. If the reduction of the pressure is increased to one-half or one-fourth of the normal, death soon follows. Eggs of the frog placed in an atmosphere of hydrogen, nitrogen, or carbon dioxide show a retarded and very irregular segmentation and development, and death within a few hours.

In the chick, it has been found that development is arrested or stopped if the oxygen supply is reduced or cut off. Even a reduction of the atmospheric pressure to 600 millimeters results in the production of small embryos, with abnormal medullary tubes, without optic vesicles or cranial flexure, and no blood capillaries, although blood islands may be present. An increase in oxygen results, in the chick, in a slightly accelerated but otherwise normal development. The most marked effect is that the skin of the embryo and all fœtal membranes become very fully charged with hæmoglobin, and so very red in color. There is no question that there is a markedly increased consumption of oxygen during the incubation period, and a corresponding production of carbon dioxide. The figures given, however, for the day-to-day oxygen consumption and carbon dioxide production come so far short of forming an ideal curve that it seems best not to do more than mention the general mass effect. There is here an opportunity for much further investigation.

7. *The Rôle of Carbon Dioxide in Development.*—Accumulations of carbon dioxide about developing eggs seem to be very generally harmful. It has been well recognized for a long time by experimental workers that thick layers of eggs at the bottom of a culture dish render development very difficult. This may be due to the fact that there is in the thickened layer a lack of oxygen, or there may be the accumulation of carbon dioxide, or both effects may occur. It was shown experimentally, by Vernon, that Echinoderm eggs subjected to an excess of carbon dioxide die very quickly. This was done by placing the eggs in a mixture of 80 per cent sea water plus 20 per cent sea water which had been saturated with carbon

dioxide. Whether the death of the eggs in this solution was due simply to the increase in the hydrogen-ion concentration has not been shown. Similar effects have been found for the eggs of Fundulus, Ctenolabrus, and of the frog.

8. *Effects of Sodium and Calcium.*—An antagonism which exists between sodium and calcium has been pointed out in another connection in this work. Each of these substances alone has upon protoplasm a poisonous effect which may be neutralized wholly, or almost wholly, by a proper proportion of the other substance. It has already been seen how this antagonism shows itself in the activity of muscle. It appears also in the processes of development. If calcium be removed from sea water, there is a modification of the behavior of eggs developing therein. The cell envelopes are altered in such a way that the cells tend to fall apart. This has been referred to in the paragraphs dealing with the isolation of blastomeres. This alteration has been interpreted by some to mean that the sodium (possibly the potassium) of the sea water is responsible for breaking down the cohesion between the cells, and that the calcium is responsible for holding them together. The sodium of the sea water—nor of any body fluid so far as is known—cannot be replaced by lithium, rubidium, or cæsium, although these elements are rather closely like sodium in their general characters.

Calcium is, of course, essential for skeletal formation. If the calcium is precipitated by sodium oxalate, embryos of the sea urchin may form spherical plutei, but without skeletons. Calcium is also necessary for the formation of cilia. An excess of calcium may lead to the formation of very large cilia upon the larvæ of many marine forms.

9. *Effects of Potassium upon Development.*—Potassium is present in the sea water—and likewise in body fluids—in only a very small amount. Notwithstanding this fact, there is a considerable accumulation of potassium within the tissues of the organism. If the small amount of potassium which is normally found in sea water be omitted, the eggs of Echinus quickly die. The eggs of Sphærechinus segment, but form only small and very turbid blastulæ. This may be due to a lack of power of the cells to absorb water, since potassium is known to be conducive to such absorption.

Additions of small amounts of potassium chloride to sea water have very important effects upon the development of eggs therein. For example, the fertilized eggs of the worm Chætopterus in an excess of potassium differentiate to the point of putting out cilia without undergoing cleavage. The eggs of Echinus or Sphærechinus in sea water to which has been added a small amount of potassium chloride (93 cubic centimeters sea water plus 7 cubic centimeters $M/20$ KCl) give rise to plutei with nearly normal guts, rudimentary skeletons, and suppressed arms. Such embryos have a tendency to be somewhat sticky and may even

fuse so as to produce double monsters. In sodium solutions and in lithium solutions similar effects upon Echinoderm eggs are obtained, though the effects are somewhat more exaggerated. Embryos grown in sodium or lithium solutions instead of forming normal gastrulæ turn the endoderm outward, thus forming what are known as exogastrulæ. This is an effect comparable to that of heat, in certain cases. Such exogastrulæ show an abundant ciliation, the skeleton structures are usually missing or greatly reduced, the embryonic arms arise in unusual positions and not in the normal numbers. Thus the symmetry of the organisms is disturbed. There is also an apparent decrease in the amount of ectodermal tissue. The degree to which this reduction takes place depends upon the period at which the exposure to the experimental solution occurs. If the exposure occurs at the time when the differentiation into ectoderm and endoderm is just beginning, the effect is much more pronounced than if it occurs at some later period. This, again, indicates the possibility that interference with some developmental phase rather than a specific chemical effect may be the cause of some of the facts observed. Herbst at first considered these abnormalities to be due to changes in osmotic conditions, but later decided that they are due to the specific effects of salt employed.

10. *The Effect of the Sulphion.*— The sulphion is probably found in the sea water in combination with sodium or magnesium. It appears to be indispensable for the proper development of Echinoderm larvæ, since in its absence the larvæ are not able to form normal plutei. It forms a natural constituent of the larval skeleton, probably in the form of calcium sulphate, and so becomes essential to the proper development of that structure. It is also found to be necessary for the normal development of the intestine, those forms which are deprived of the sulphion having a straight and undivided gut instead of one which is divided and bent, and, in Sphærechinus, the gut may be evaginated. The sulphion is also of importance in the formation of pigment, and serves to prevent the hypertrophy of cilia in the larvæ of Echinus, a process which is strongly promoted by an excess of calcium.

11. *Effects of Combinations of Salts.*—A very striking effect of modification of the external medium is seen in the results presented by Loeb on twin production. He found that if the eggs of Strongylocentrotus are fertilized in normal sea water and then placed in neutral solutions of:

KCl plus $CaCl_2$, or
KCl plus NaCl, or
NaCl plus $CaCl_2$, or
NaCl plus $MgCl_2$, and

left for from $\frac{1}{2}$ to 1 hour after they have reached the two-cell stage, and then transferred to sea water, from 50 to 90 per cent of the embryos will produce twins. Here, of course, the normal balance of the mole-

cules is very much upset, although the total concentration, and, therefore, the osmotic pressure, may be normal.

Internal Factors of Development.—There have been presented illustrative examples of the influence of some of the external factors which may have a modifying or determining influence upon development. It now remains to say just a word concerning some of the *internal factors* which influence development.

Much effort has been expended in the attempt to discover something of the nature of the internal factors, the method in which they operate, and the effects produced. Even now our knowledge concerning them is exceedingly fragmentary and almost wholly confined to the effects produced. It must be confessed that there is no information as to the chemical nature of the substances involved, or of the chemical reactions

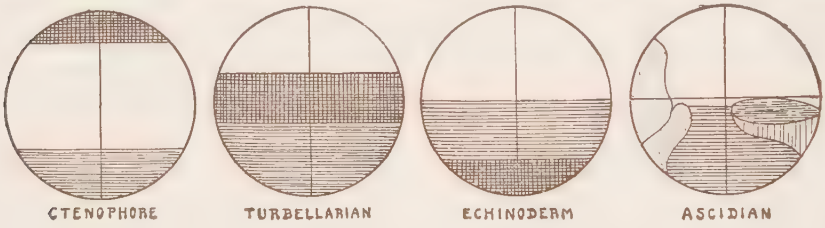


FIG. 148.—Eggs of various animals showing patterns assumed by the materials which give rise to the various body regions. In the Ctenophore, Turbellarian, and Echinoderm, the egg has undergone division, and the plasmas becoming ectoderm, mesoderm, and endoderm are represented in clear-white, cross-hatching, and parallel rulings, respectively. In the Ascidian egg two divisions have occurred, and several definitely arranged substances are distinguishable. (*After Conklin*, 1915.)

which occur as incidents in the direction of developmental processes. Some knowledge has been gained concerning the *promorphology* of the egg. It has been demonstrated that in many cases the egg is not a perfectly homogeneous structure, any portion of which may give rise to any portion of an embryo, as has been claimed by some biologists. On the contrary, the egg is a very heterogeneous structure, and there have been found in the cytoplasms of some eggs certain substances, *formative substances*, which represent, in a chemical way, certain differentiated regions or structures of the embryo. So, with the eggs of some of the Echinoderms, it is possible, even before cleavage has occurred, to predict that ectoderm and structures arising from it will arise from that half of the egg extending from the animal pole nearly to the equator of the egg. Similarly, the endoderm and the structures to be derived from it will arise from the mass of material extending from the equatorial region to within a short distance of the vegetative pole. The mesogloea and the structures to be derived from it will arise from the material which forms the polar cap at the vegetative pole of the egg.

Conklin has shown that, in the egg of the Ascidian Styela, there are in the cytoplasm four or five kinds of substance, definitely arranged,

and definitely distributed during the early cleavages, each of which gives rise to a definite region of the embryo. He recognizes these substances by their color reactions after staining with hæmatoxylin and picric acid, though it is possible to determine their presence in the unstained, living egg, by close observation. Conklin has found that (Fig. 148):

The yellow substance gives rise to muscle and mesoderm.

The gray substance gives rise to nervous system and notocord.

The slate-blue substance gives rise to endoderm.

The colorless substance gives rise to ectoderm.

It is possible by means of centrifugal force to give to these substances an abnormal stratified arrangement, for they have different specific gravities. Eggs in which the formative substances have been so stratified go through the cleavage stages, but do not produce normal embryos. Their organs are variously misplaced, depending upon the degree to which the formative substances have been thrown out of their normal distribution.

These cases cited, and others as well, point to the fact that the characteristics of general organization of an embryo depend upon the presence in the cytoplasm of definite formative substances. It should not be inferred that there are present in the egg at the onset of development all of the substances which may be needed in the direction of development. It seems likely that, as cell divisions go on, and as the chemical reactions characteristic of its kind occur in the embryo, there are from time to time produced here and there in the cells of the embryo substances which may influence the direction of development of nearby cells. As an example of this, the case referred to by McBride may be cited. In the larva of the sea urchin, Echinus, a single hydrocœl on the left side is formed during the normal development. The ectoderm lying adjacent to this rudiment of the water-vascular system becomes invaginated and forms a hollow sac, the amniotic sac.

From the floor of this sac develop the adult nerve ring and spines. In the center of the floor an invagination is formed which gives rise to the adult mouth. From the peritoneal epithelium of the left cœlomic sac, which lies beneath the hydrocœl, are developed the dental pockets and the teeth which project into them.

But, occasionally, a second hydrocœl is developed which is situated on the right side of the larva. When this takes place the ectoderm of the *right side* of the body becomes invaginated to form a deep sac, from the floor of which a second nerve ring, a second set of adult spines, and a second mouth are developed, whilst the underlying peritoneal epithelium of the *right cœlomic sac* gives rise to a second set of dental pockets.

Now, although we have good reason to believe that the common free-swimming ancestor of all Echinoderms possessed two hydrocœls, yet it is as certain as anything can be that this ancestor did not possess two mouths surrounded by nerve rings, nor did it have the highly differentiated spines and teeth of the Echinoid. The production of a second hydrocœl is probably an ancestral

reminiscence, but the production of these other organs cannot possibly be so explained.

The only conclusion, therefore, that we can draw is that, in the natural development of Echinus, the hydrocœl emits a hormone which caused the ectoderm to form the amniotic invagination, and which caused the left cœlomic vesicle to give rise to the dental pockets. It follows further that particular portions of ectoderm and of cœlomic wall are not specialized so that they alone can undergo these modifications, but that any portion of the ectoderm and any portion of the cœlomic wall can undergo similar modifications, provided that they are acted upon by the appropriate hormone, as is shown by the effect of the right hydrocœl upon the right cœlomic sac and on the ectoderm of the right side. Development is then a kind of progressive specialization, due to the influence of one organ on another by means of hormones.

A somewhat similar instance is that of the influence of the optic cup, in the embryo of a salamander, in bringing about the development of the lens. The lens arises from the ectoderm which normally covers the optic cup, which is itself derived from an outgrowth of the forebrain. If the original ectoderm be removed from its position over the optic cup just before the time when differentiation of a lens would normally occur, and there be substituted for it ectoderm taken from any other region of the body, the final result is the same. It makes no difference from what portion of the body the ectoderm is taken, a lens will be formed, provided the substitution is made at the appropriate time. Also, the rudiment of the optic cup may be dissected out from its normal position and be grafted into the body at almost any position. The ectoderm covering the cup always forms a lens. This can be satisfactorily explained only upon the hormone hypothesis.

The Chromosomes.—A second group of chemical agents which are of the utmost importance in development are the chromosomes contained within the nucleus of the egg. Knowledge of these structures is very limited in spite of the vast amount of labor which has been expended upon them. Something is known of their number, size, shape, staining properties, behavior in mitosis, and that they are primarily responsible for the determination of sex and of the details of biparental inheritance. Nothing is known of their chemical nature as individuals, but from their effects it is judged that they must differ from each other chemically. There is no information as to the manner in which any parental character is transmitted from cell to cell. There is evidence that the various determiners of characters occupy definite positions upon definite chromosomes. In fact, the evidence along this line is so definite that Morgan and his associates have mapped the location of 125 or more characters upon four chromosomes of Drosophila (see Fig. 149). This figure is introduced simply as evidence of the fact that hereditary transmission involves, as its instrument, the use of a well-established chemical framework. It is impossible here to go into the details of inheritance through the medium of the chromosomes. That is a problem for another course.

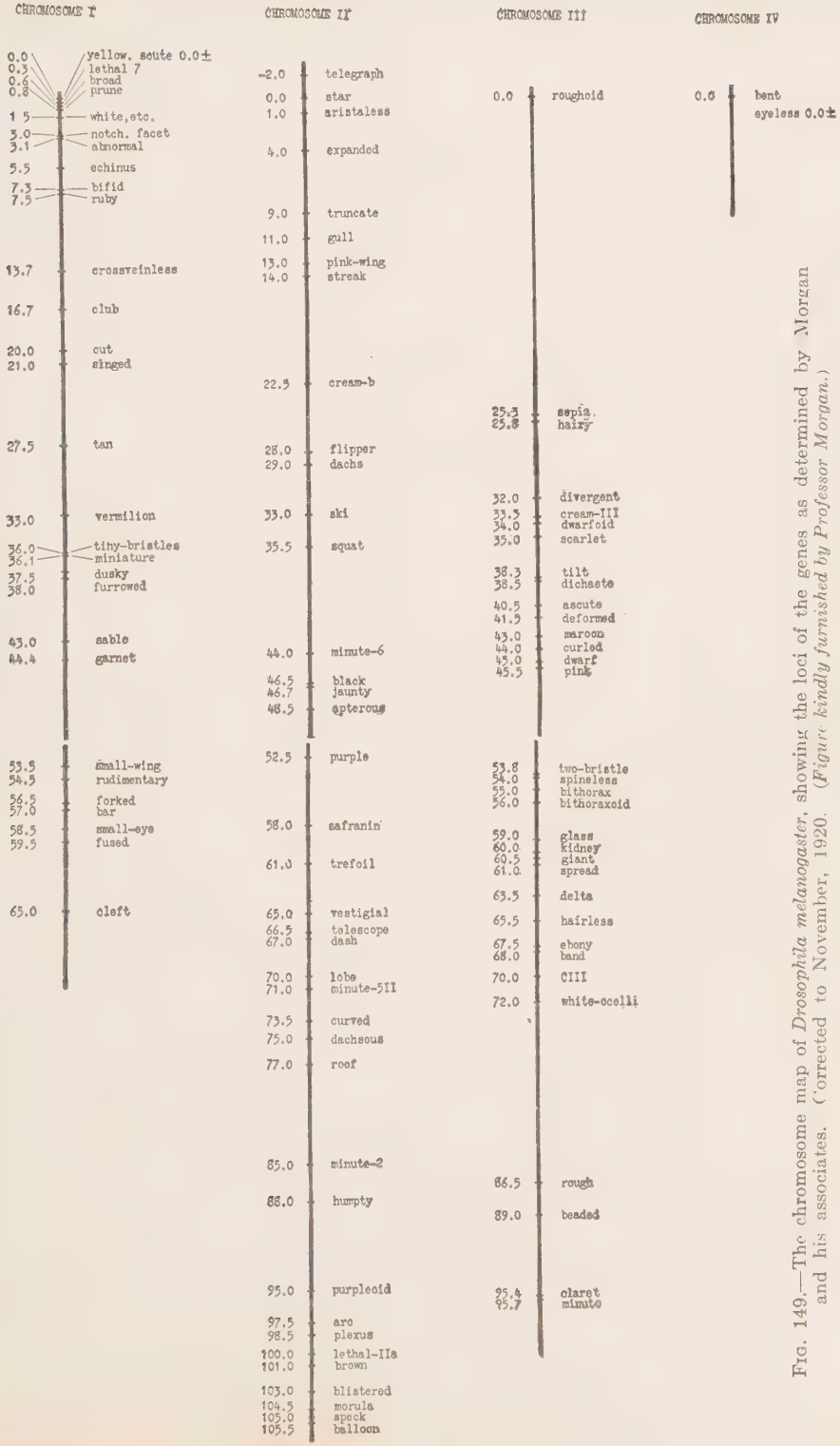


FIG. 149.—The chromosome map of *Drosophila melanogaster*, showing the loci of the genes as determined by Morgan and his associates. Corrected to November, 1920. (*Figure kindly furnished by Professor Morgan.*)

BIBLIOGRAPHY

Abderhalden, Emil, 1914, Defensive Ferments of the Animal Kingdom, William Wood and Company, New York.

Adams, A., 1912, Effects of Atmospheres Enriched with Oxygen upon Living Organisms, *Biochem. J.* **6,** 297.

Adrian, E. D., 1912, Conductivity of Subnormal Disturbance, *J. Physiol.*, **45,** 389.

1913, Wedensky Inhibition, *J. Physiol.*, **46,** 384.

1914, All-or-none Principle in Nerve, *J. Physiol.*, **47,** 460.

1914, Temperature Coefficient of Refractory Period in Nerve, *J. Physiol.*, **48,** 453.

1914, Relation between Size of Propagated Disturbance and Rate of Conduction in Nerve, *J. Physiol.*, **48,** 53.

1915, Recovery of Conductivity and of Excitability in Nerve, *J. Physiol.*, **50,** 345.

———, and K. Lucas, 1912, Summation of Propagated Disturbances in Muscle and Nerve, *J. Physiol.*, **44,** 68.

Alder, Gustawa, 1917, Oxygen Tension in the Tissues of Certain Invertebrates, *Scan. Arch. Physiol.*, **35,** 144.

Aldrich, T. B., 1912, On Feeding Young Pups the Anterior Lobe of the Pituitary Gland, *Am. J. Physiol.*, **30,** 352.

1912, On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland, *Am. J. Physiol.*, **31,** 94.

1913, The Iodine Content of the Small, Medium, and Large Thyroid Glands of Sheep, Beef, and Hogs, *Orig. Com. Eighth Intern. Cong. App. Chem.*, **19.**

Alexandrowicz, S., 1913, Beiträge zur Vergleichenden Physiologie der Verdauung, *Pflüger's Arch. ges. Physiol.*, **150,** 57–86.

Allen, Bennet Mills, 1925, The Effects of Extirpation of the Thyroid and Pituitary Glands upon the Limb Development of Anurans, *J. Exptl. Zoöl.*, **42,** No. 1.

Allen, G. D., 1919, Studies on the Respiratory Metabolism of Planaria, *Am. J. Physiol.*, **48,** 93.

Alsberg, C. L., 1915, Note on Reduction of Oxyhaemocyanin in Serum of *Limulus*, *J. Biol. Chem.*, **23,** 495.

———, and W. M. Clark, 1914, The Solubility of Oxygen in the Serum of *Limulus polyphemus* and in Solutions of Pure *Limulus* Haemocyanin, *J. Biol. Chem.*, **19,** 503–510.

Alvarez, W. C., and Esther Starkweather, 1919, Conduction in Small Intestine, *Am. J. Physiol.*, **50,** 252.

Andrews, E. A., 1914, Secretion in Protozoa, *Science,* N. S., **39,** 472.

Andrus, E. Cowles, 1919, Alterations in the Activity of the Terrapin's Heart Relative to Slight Changes in the pH Value of the Perfusate, *Am. J. Physiol.*, **48,** No. 2.

———, and E. P. Carter, 1922, The Effect upon the Cold-blooded Heart of Changes in the Ionic Content of the Perfusate, *Am. J. Physiol.*, **59,** No. 1.

Arey, L. B., 1917, Normal Shape of Mammalian Red Blood Corpuscles, *Am. J. Anatomy,* **22,** 439.

Armstrong, E. F., 1904 (1), The Rate of Change Conditioned by Sucroclastic Enzymes and Its Bearing on the Law of Mass Action, *Proc. Roy. Soc. London,* **73,** 500.

1904 (2), The Influence of the Products of Change on the Rate of Change Conditioned by Sucroclastic Enzymes, *Proc. Roy. Soc. London,* **73,** 516.

Armstrong, H. E., 1913, The Nature of Enzymes and Their Action as Hydrolytic Agents, *Proc. Roy. Soc. London*, **86B**, 359.

Arnold, G., 1909, Intra-cellular and General Digestive Processes in Planaria, *Quart. J. Mic. Sci.*, **54**, 207.

Arrhenius, Svante, 1914, *Faraday Lecture*—The Theory of Electrolytic Dissociation, *Trans. Chem. Soc.*, **105**, 1414.

Atwater, W. O., and F. G. Benedict, 1899, Metabolism of Matter and Energy in the Human Body, *U. S. Dept. Agr., Bull.* 109.

Babak, E., 1910, Development of Surface in Organisms and Its Adaptability, *Biol. Cent.*, **30**, Nos. 7 and 8, through *Chem. Abstracts*, **4**, 2691.

1913, Regulation of Respiratory Stream in Lamellibranchs, *Z. allgem. Physiol.*, **15**, 184–198.

——, and B. Dedek, 1907, Initial Stimulus to Respiratory Movements in Fishes, *Pflüger's Arch. ges. Physiol.*, **119**, 483.

——, and Ot. Foustka, 1907, Respiratory Stimulus with Arthropoda, *Pflüger's Arch. ges. Physiol.*, **119**, 530.

Bachmann, G., 1916, The Interauricular Time Interval, *Am. J. Physiol.*, **41**, 309.

Bacon, R. F., 1909, Excreta of Python, *Philippine J. Sci.*, **49**, 165.

Badertscher, J. A., 1924, Results Following the Extirpation of the Pineal Gland in Newly Hatched Chicks, *Anat. Record*, **28**, No. 3.

Baldi, E., 1922, Physiology of the Nervous System of Insects and Circus Movements in Coleoptera, *J. Exptl. Zoöl.*, **36**, No. 3.

Bancroft, F. W., and C. O. Esterley, 1903, A Case of Polarization in the Ascidian Heart, *Univ. Calif. Pub. Zoöl.*, **1**, 105.

Bancroft, W. D., 1921, Applied Colloid Chemistry, McGraw-Hill Book Company, Inc., New York.

Banting, F. G., C. H. Best, J. B. Collip, W. R. Campbell, A. A. Fletcher, J. J. R. Macleod, and E. C. Noble, 1922, The Effect Produced on Diabetes by Extracts of Pancreas, *Trans. Assoc. Am. Physicians.*

Barcroft, Joseph, 1908, Zur Lehre vom Blutgasvechsel in den verschiedenen Organen, *Ergebnisse Physiol.*, **7**, 699.

1914, The Respiratory Function of the Blood, 320 pp., Cambridge University Press.

1924, The Significance of Haemoglobin, *Physiol. Rev.*, **4**, No. 3.

Barrell, Joseph, 1916, Influence of Silurian-Devonian Climates on the Rise of Air-breathing Vertebrates, *Bull. Geol. Soc. Am.*, **27**, 387.

Barrett J. O. W., and G. Arnold, 1910, Study of Blood of Certain Coleoptera, *Quart. J. Med. Sci.*, N. S., **56**, 149.

Bayliss, W. M., 1908, On Reciprocal Innervation in Vaso-motor Reflexes and on the Action of Strychnine and of Chloroform Thereon, *Proc. Roy. Soc. London*, **80B**, 339.

1909, The Ostmotic Pressure of Congo Red and of Some Other Dyes, *Proc. Roy. Soc. London*, **81B**, 269.

1911, On Adsorption as Preliminary to Chemical Reaction, *Proc. Roy. Soc. London*, **84B**, 81.

1912, Researches of the Nature of Enzyme Action, II. The Synthetic Properties of Anti-emulsin, *J. Physiol.*, **43**, 455.

1913, Researches on the Nature of Enzyme Action, III. The Synthetic Action of Enzymes, *J. Physiol.*, **46**, 236.

1913, The Nature of Enzyme Action, 3d. ed., Longmans, Green & Co., London.

1915, The Nature of Enzyme Action, The Action of Insoluble Enzymes, *J. Physiol.*, **50**, 85.

1919, The Neutrality of the Blood, *J. Physiol.*, **53**, 162.

1920, Principles of General Physiology, 3d ed., Longmans, Green & Co., London.

1920, Reversible Gelation in Living Protoplasm, *Proc. Roy. Soc. London*, **91B,** 196.

Bechhold, H. 1919, Colloids in Biology and in Medicine, translated by Jesse G. M. Bullowa, D. Van Nostrand Company, New York.

Benedict, S. R., 1905, Rôle of Certain Ions in Rhythmic Heart Activity, *Am. J. Physiol.*, **13,** 192.

1908, The Influence of Salts and Non-electrolytes upon the Heart, *Am. J. Physiol.*, **22,** No. 1.

Berkeley, Earl of, and E. G. J. Hartley, 1916, Further Determinations of Direct Osmotic Pressure, *Proc. Roy. Soc. London*, **92A,** 477.

Bert, Paul, 1878, La Pression Barometrique, 1168 pp., Masson, Paris.

1882, Sur la Richesse en hemoglobine du sang des animaux vivants sur les hauts lieux, *Compt. rend.*, **94,** 805.

Bethe, Albrecht, 1897, Das Nerve system von *Carcinus Maenas*, *Arch. Mikr. Anat. u. Entwickl. Mech.*, **50,** 460, and **51,** 382.

1903, Allgemeine Anatomie und Physiologie des Nervensystems, 487 pp., Thieme, Leipzig.

1908, Die Bedeutung der Elektrolyten für die rhythmischen Bewegungen der Medusen, I. Die Wirkung der im Seewasser enthaltenen Salze auf die normale Meduse, *Pflüger's Arch. ges. Physiol.*, **124,** 541.

1909, II. Ausgriffspunkt der Salze, Einfluss der Anionen, OH und H ionen, *Pflüger's Arch. ges. Physiol.*, **127,** 219.

Biedermann, W., 1911, Die Aufnahme, Verarbeitung, und Assimilation der Nahrung, *Winterstein's Handbuch Verg. Physiol.*, **2.**

Birge, E. A., and C. Juday, 1908, Gases Dissolved in Wisconsin Lakes, *Bull. U. S. Bur. Fish.*, **28.**

1912, A Limnological Study of the Finger Lakes of New York, *Bull. U. S. Bur. Fish.*, **32.**

Bloor, W. R., 1916, Fat Assimilation, *J. Biol. Chem.*, **24,** 447.

1923, Perfusion Fluid and the Heart of the Summer Frog, *Physiol. Abstracts*, **3.**

Botazzi, F., 1897, La pression osmotique du sang des animaux marins, *Arch. ital. biol.*, **28,** 61.

1902, Digestive System of Crayfish, *Z. Biol.*, **43,** 363.

1908, Osmotische Druch und elektrische Leitfähigkeit der Flussigkeiten der einzeligen, pflanzliche und tierische Organismen, *Ergebnisse Physiol.*, **7,** 161.

1916, Nouvelles Recherches sur les Muscles stries et sur les Muscles lisse d'animaux homeothermes, *Arch. ital. biol.*, **65,** 17.

Bowman, W., 1842, On the Structure and Use of the Malpighian Bodies of the Kidney, with Observations on the Circulation Through That Gland, *Phil. Trans. Roy. Soc. London*, Pt. I, 57.

Bradley, H. C., and H. S. Gasser, 1911, Intestinal Absorption, *Proc. Am. Soc. Biol. Chem.*, **9.**

Brodie, T. G., 1914, *Croonian Lecture*—A New Conception of the Glomerular Function, *Proc. Roy. Soc. London*, **87B,** 571.

Brown, Charles W., and F. J. Morgan, 1918, An Interpretation of the Cycles of Carbon, Nitrogen, and Sulphur, *Abstracts Bact.*, **2,** 2.

Budington, R. A., 1902, Some Physiological Characteristics of Annelid Muscle, *Am. J. Physiol.*, **7,** No. 1.

1919, The Influence of Certain Gland Substances on the Growth of Plant Tissues, *Biol. Bull.*, **37,** No. 3.

———, and Helen F. Harvey, 1915, Division Rate in Ciliate Protozoa as Influenced by Thyroid Constituents, *Biol. Bull.*, **28,** No. 5.

Buglia, G., 1908, Gas Exchange of Eggs of *Aplysia* in Different Stages of Development, *Arch. Physiol.*, **5,** 455.

Bullot, G., 1904, Artificial Parthenogenesis and Regular Segmentation in an Annelid (*Ophelia*), *Univ. Calif. Pub. Phys.*, **1,** 165.

1904, On the Toxicity of Distilled Water for the Fresh-water *Gammarus*—Suppression of This Toxicity by the Addition of Small Quantities of Sodium Chloride, *Univ. Calif. Pub. Phys.*, **1.**

Burrows, M. T., 1912, Rhythmical Activity of Isolated Heart Muscle Cells in Vitro, *Science*, N. S., **36,** 916.

1918, A Note on the Mechanism of Heart Muscle Contraction, *Am. J. Physiol.*, **45,** 556.

Buytendyk, F. J. J., 1909, On the Consumption of Oxygen by Cold-blooded Animals in Connection with Their Size, *Proc. Akad. Wet.*, **12,** 48.

Cannon, W. B., 1911, The Mechanical Factors of Digestion, Edward Arnold and Company, London.

1915, Bodily Changes in Fear, Hunger, Pain, and Rage. D. Appleton & Ampersand Company, New York.

1918, Physiological Basis of Thirst, *Proc. Roy. Soc. London*, **90B.**

———, and McKeen Cattell, 1916, The Influence of the Adrenal Secretion on the Thyroid, *Am. J. Physiol.*, **41,** 74.

———, and L. B. Nice, 1913, The Effect of Adrenal Secretion upon Muscular Fatigue, *Am. J. Physiol.*, **32,** 44.

Carlson, A. J., 1904, Contributions to the Physiology of the California Hagfish, *Z. allgem. Physiol.*, **4.**

1904, The Nervous Origin of the Heart Beat in Limulus, and the Nervous Nature of Coordination or Conduction in the Heart, *Am. J. Physiol.*, **12,** No. 1.

1904, The Rhythm Produced in the Resting Heart of Molluscs by the Stimulation of the Cardio-accelerator Nerves, *Am. J. Physiol.*, **12,** No. 1.

1905, Cardiac Nerves in Arthropods, *Am. J. Physiol.*, **15,** 127.

1905, Comparative Physiology of the Invertebrate Heart, II. Cardiac Nerves in Molluscs, *Am. J. Physiol.*, **13,** 396.

1905, Comparative Physiology of the Invertebrate Heart, III. The Cardiac Nerves of Molluscs, *Am. J. Physiol.*, **14,** 16.

1905, Fluidity of Conducting Substance in Nerve, *Am. J. Physiol.*, **13,** 351.

1905, Osmotic Pressure and Heart Activity, *Am. J. Physiol.*, **15,** 357.

1905, Rate of Conduction in a Motor Nerve, *Am. J. Physiol.*, **15,** 136.

1905, Temperature and Heart Activity with Special Reference to the Heat Stand Still, *Am. J. Physiol.*, **15,** 207.

1906, Chemical Conditions for Heart Activity, *Am. J. Physiol.*, **16,** 378.

1906, Heart Rhythms under Normal and Experimental Conditions, *Am. J. Physiol.*, **16,** 47.

1906, On the Cause of the Cessation of Rhythm in Isotonic Solutions of Non-electrolytes, *Am. J. Physiol.*, **16,** 221.

1906, The Excitability of the Heart During the Different Phases of the Heart Beat. *Am. J. Physiol.*, **16,** 67.

1906, The Inhibitory Effects of the Single Induced Shock, *Am. J. Physiol.*, **16,** 100.

1906, The Presence of Cardio-regulative Nerves in the Lampreys, *Am. J. Physiol.*, **16,** 230.

1906, The Relation between Intensity of the Stimulus and the Magnitude of the Contraction, *Am. J. Physiol.*, **16,** 85.

1908, Pulsating Vessels in Worms, *Am. J. Physiol.*, **22,** 353.

1911, Effects of Stretching Nerve on Rate of Conduction, *Am. J. Physiol.*, **27,** 323.

CATHCART, E. P., 1921, The Physiology of Protein Metabolism, Longmans, Green & Co., London.

CHAMBERLIN, T. C. and R. T., 1908, Early Terrestrial Conditions That May Have Favored Organic Synthesis, *Science*, N. S., Dec. 25, 1908.

CHAMBERS, ROBERT, 1915, Microdissection Studies on the Physical Properties of Protoplasm, *Lancet-Clinic*, March, 1915.

1917, Microdissection Studies, *Anat. Record*, **2,** No. 6.

1917, The Visible Structure of Cell Protoplasm and Death Changes, *Am. J. Physiol.* **43,** No. 1.

1919, Some Studies on the Surface Layer in the Living Egg Cell, *Proc. Soc. Exptl. Biol. Med.*, **17,** 41.

1920, Dissection and Injection Studies on the Amoeba, *Proc. Soc. Exptl. Biol. Med.*, **18,** 66.

1921, A Simple Apparatus for Micro-manipulation under the Highest Magnifications on the Microscope, *Science*, N. S., **54,** 411.

1921, A Simple micro-injection Apparatus Made of Steel, *Science*, N. S., **54,** 552.

1921, Some Problems in the Maturation and Fertilization of the Egg of the Echinoderm, *Biol. Bull.*, **41,** No. 6.

1921, Studies on the Organization of the Starfish Egg, *J. Gen. Physiol.*, **4,** 41.

1921, The Effect of Experimentally Induced Changes in Consistency on Protoplasmic Movement, *Proc. Soc. Exptl. Biol. Med.*, **19,** 87.

1922, A Micro-injection Study of the Permeability of the Starfish Egg, *J. Gen. Physiol.*, **5,** 189.

1922, New Apparatus and Methods for the Dissection and Injection of Living Cells, *Anat. Record*, **24,** No. 1.

———, and HIROSHE' OHSHIMA, 1922, Merogony Experiments on Sea Urchin Eggs, *Proc. Soc. Exptl. Biol. Med.*, **19,** 320.

———, and G. S. RENYI, 1925, The Structure of the Cells in Tissue as Revealed by Micro-dissection, *Am. J. Anatomy*, **35,** No. 3.

CHILD, C. M., 1915, A Dynamic Conception of the Individual Organism, *Proc. Nat. Acad. Sci.*, **1,** 164.

1919, Carbon Dioxide Production During Starvation in Planaria, *Am. J. Physiol.*, **48,** 231.

1919, Effect of Cyanide on Carbon Dioxide Production and on Susceptibility to Lack of Oxygen in Planaria, *Am. J. Physiol.*, **48,** 372.

1919, The Axial Gradients in the Hydrozoa, I, *Biol. Bull.*, **36,** No. 3. 1919, II, *Biol. Bull.*, **37,** No. 2. 1921, IV, *Biol. Bull.*, **41,** No. 2. 1923, V, *Biol. Bull.*, **45.**

CHRISTIANSEN, J., C. G. DOUGLASS, and J. S. HALDANE, 1914, The Absorption and Dissociation of Carbon Dioxide by Human Blood, *J. Physiol.*, **48,** 245.

CHURCHILL, E. P., 1915, Absorption of Fat by Mussels, *Science*, N. S., **41,** 470.

1916, The Absorption of Nutriment from Solutions by Fresh-water Mussels, *J. Exptl. Zoöl.*, **21,** 403.

CLARK, E. L., 1914, Early Pulsation of Lymph Hearts in Chick Embryos, *J. Exptl. Zoöl.*, **17,** 373; *Anat. Record*, **9,** 65.

———, and E. R., 1925, The Development of Adventitial (Rouget) Cells on the Blood Capillaries of Amphibian Larvae, *Am. J. Anatomy*, **35,** No. 2.

CLARK, W. M., 1920, Determination of Hydrogen Ions, Williams and Wilkins Company, Baltimore.

CLOWES, G. H. A., 1916, Protoplasmic Equilibrium, *J. Phys. Chem.*, **20,** 407.

COGHILL, G. E., 1915, Preliminary Studies on Intracellular Digestion and Assimilation in Amphibian Embryos, *Science*, N. S., **42,** 347.

COHN, E. J., 1916, Hydrogen Ion Concentration and Spermatozoa, *Anat. Record*, **11,** 530.

1925, The Physical Chemistry of the Proteins, *Physiol. Rev.*, **5,** No. 3.

Cohnheim, Otto, 1912, Enzymes, John Wiley & Sons, Inc., New York.

Cole, William H., 1926, Temperature and Locomotion in Planaria, *J. Gen. Physiol.*, **9,** 503.

Collip, J. B., 1920, Effect of Dilution on the Osmotic Pressure and the Electrical Conductivity of Whole Blood, Blood Serum, and Corpuscles, *J. Biol. Chem.*, **42,** No. 1.

1920, Maintenance of Osmotic Pressure within the Nucleus, *J. Biol. Chem.*, **42,** No. 1.

1920, Osmotic Pressure of Tissue as Determined by the Cryoscopic Method, *J. Biol. Chem.*, **42,** No. 1.

1920, Studies on Molluscan Coelomic Fluid, *J. Biol. Chem.*, **45,** No. 1.

1920, The Alkali Reserve of Marine Fish and Invertebrates, *J. Biol. Chem.*, **44,** No. 2.

1921, A Reversal of Depressive Action of Small Doses of Adrenalin, *Am. J. Physiol.*, **55,** No. 3.

1923, Glucokinin: A New Hormone Present in Plant Tissue, *J. Biol. Chem.*, **56,** No. 2.

1923, Glucokinin, *J. Biol. Chem.*, **58,** No. 1.

1923, The History of the Discovery of Insulin, *Northwest Medicine*, August, 1923.

Coombs, Helen, and F. H. Pike, 1918, The Rôle of Afferent Impulses in the Control of Respiratory Movements, *Proc. Soc. Exptl. Biol. Med.*, **15,** 55, and *Am. J. Physiol.*, **45,** 569.

Cooper, Sybil, 1926, The Conduction of a Nervous Impulse in a Narcotized Region of Nerve, *J. Physiol.*, **61,** 305.

Craifaleanu, A. D., 1916, Studies on the Ferments of Marine Animals, *Pub. della Staz. Zoöl. de Napoli*, **1,** No. 1, 155 and **30,** 3–19.

1918, Proteolytic Enzymes in Liver of Sepia, *Chem. Abstracts*, **12,** 2385.

Crider, J. O., and W. W. Robinson, 1916, The Effect of Certain Procedures upon the Course of Fatigue in Frog's Muscle, *Am. J. Physiol.*, **43.**

Crozier, W. J., 1916, The Rhythmic Pulsations of the Cloaca of Holothurians, *J. Exptl. Zoöl.*, **20.**

1918, On Sensory Activation by Alkalies, etc., *Am. J. Physiol.*, **45,** No. 4.

1918, The Amount of Bottom Material Ingested by Holothurians, *Chem. Abstracts*, **12.**

1926, The Distribution of Temperature Characteristics for Biological Processes, *J. Gen. Physiol.*, **9,** 531.

———, and T. J. B. Steer, 1926, On the Modification of Temperature Characteristics, *J. Gen. Physiol.*, **9,** 547.

———, W. B. Rogers, and B. I. Harrison, 1915, Methods Employed in Determining the Hydrogen Ion Concentration in Body Fluids, *Surgery, Gynecol., and Obstetrics*, 722–727.

Cuenot, L., 1907, Absorbing and Excreting Functions of the Liver of Cephalopods, *Arch. Zoöl. Exp.* (4), **7,** 227.

1913, Excretion and Phagocytosis in Sipunculids, *C. R. Soc. Biol., Paris*, **74,** 159.

1914, Les Organes Phagocytaires des Mollusques, *Arch. Zoöl. Exp.*, **54,** 267.

Cunningham, J. T., 1921, Hormones and Heredity, Constable and Co., London.

Curtis, W. C., 1923, Current Aspects of the Doctrine of Organic Evolution, *School and Society*, **17,** 393.

Cushing, Harvey, 1912, The Pituitary Body and Its Disorders, J. B. Lippincott Company, Philadelphia.

Cushny, A. R., 1917, The Secretion of Urine, Longmans, Green & Co., London.

Dahlgren, U., and W. Kepner, 1908, The Principles of Animal Histology, The Macmillan Company, New York.

Dakin, W. J., 1908, The Osmotic Concentration of the Blood of Fishes Taken from Sea Water of Naturally Varying Concentration, *Biochem. J.*, **3,** 258.

DALE, DOROTHY, and C. R. A. THACKER, 1914, Hydrogen Ion Concentration Limiting Automaticity in Different Regions of the Frog's Heart, *J. Physiol.*, **47,** 493.

DALLWIG, H. C., A. C. KOLLS, and A. S. LOEVENHART, 1915, The Mechanism Adapting the Oxygen Capacity of the Blood to the Requirements of the Tissues, *Am. J. Physiol.*, **39,** 77.

DAWSON, A. B., 1920, The Intermuscular Nerve Cells of the Earthworm, *J. Comp. Neurol.*, **32,** No. 2.

DAY, E. C., 1919, The Physiology of the Nervous System of the Tunicate, *J. Exptl. Zoöl.*, **28,** No. 2.

1921, The Physiology of the Nervous System of the Tunicate, *J. Exptl. Zoöl.*, **34,** No. 1.

DHERE, CH., 1916, Recherches sur l'Hemocyanine, *J. physiol. path. gén.*, **16,** 985, and **18,** 221.

DIXON, W. E., 1905, The Clioscribe, an Instrument for Recording Ciliary Activity, *J. Physiol.*, **32,** 395.

DOUGLASS, C. G., 1910, Determination of the Total Oxygen Capacity and Blood Volume at Different Altitudes by the Carbon Monoxide Method, *J. Physiol.*, **40,** 472.

1910, Periodic Breathing at High Altitudes, *J. Physiol.*, **40,** 454.

DREW, G. A., 1908, Physiology of the Nerve System of the Razor Shell Clam, *J. Exptl. Zoöl.*, **5,** 311.

DREW, G. H., 1910, Some Points in the Physiology of Lamellibranch Blood Corpuscles, *Quart. J. Med. Sci.*, **54,** 605.

DUBOIS, D. and E. F., 1916, A Formula to Estimate the Approximate Surface Area If Height and Weight Are Known, *Arch. Internal Med.*, **17.**

DYSEK, V., 1914, Regulation of Respiratory Movements in Hibernating Animals, *Zentr. Biochem. Biophysk.*, **15,** 924, abstract in *Chem. Abstracts*, **8,** 1916.

EDDY, NATHAN B., 1921, A Simple Device for the Demonstration of Heart Block in the Student Laboratory, *J. Lab. Clin. Med.*, **6.**

———, and A. W. DOWNS, 1921, Extensibility of Muscle: Production of Carbon Dioxide When It Is Made to Support a Weight, *Am. J. Physiol.*, **56,** No. 1.

1924, The Action of Preparations of the Endocrine Glands upon the Work Done by Skeletal Muscle, *Am. J. Physiol.*, **69,** No. 2.

EDWARDS, J. G., and H. S. FORGRAVE, 1923, The Rate of Locomotion of Amoeba in Alkali Chlorides, *Johns Hopkins Hosp. Bull.*, **34,** 387.

———, and E. K. MARSHALL, 1924, Microscopic Observations of the Living Kidney after Injection of Phenolsulphonephthalein, *Am. J. Physiol.*, **70,** No. 3.

EFFRONT, JEAN, 1902, Enzymes and Their Applications, John Wiley & Sons, Inc., New York.

ESTERLY, C. O., 1916, The Feeding Habits and Food of Pelagic Copepods and the Question of Nutrition by Organic Substances in Solution in the Water, *Univ. Calif. Pub. Zoöl.*, **16,** No. 14.

EVANS, C. L., 1926, The Physiology of Plain Muscle, *Physiol. Rev.*, **6,** No. 2.

EVANS, T. J., 1913, Organisms of Brine Cultures, *Brit. Assoc. Advancement Sci., Eighty-third Rept.*, 530.

EWART, A. J., 1903, On the Physics and Physiology of Protoplasmic Streaming in Plants, The Clarendon Press, Oxford.

EYSTER, J. A. E., and W. J. MEEK, 1921, The Origin and Conduction of the Heart Beat, *Physiol. Rev.*, **1,** 1.

FALLOISE, A., La Digestion chez Cephalopods, *Arch. intern. physiol.*, **3,** 282.

FARMER, C. J., 1917, A Method for the Preparation of Uniform Collodion Membranes, *J. Biol. Chem.*, **32.**

Faust, E. C., 1921, A Study and Development of the Excretory System in a Cysticercous Larva, *Cercaria pekinensis*, *Parasitology*, **13,** No. 3.

Feldmann, W. M., 1912, Method for Calculating the Number of Capillaries Filled with Blood in Human Body, *J. Physiol.*, **43,** 35.

Fenn, W. O., 1916, Salt Antagonism in Gelatine, *Proc. Nat. Acad. Sci.*, **2,** 534.

1916, Similarity of Behavior of Protoplasm and Gelatine, *Proc. Nat. Acad. Sci.*, **2,** 539.

1918, The Effects of Acids and Salts on the Precipitation of Gelatine by Alcohol, *J. Biol. Chem.*, **33,** No. 2.

1918, The Effects of Mixtures of Salts on the Precipitation of Gelatine by Alcohol, *J. Biol. Chem.*, **34,** No. 1.

1918, The Effect of Salts on the Precipitation of Acid and Alkaline Gelatine by Alcohol, *J. Biol. Chem.*, **33,** No. 3.

1918, Precipitation of Gelatine by Mixtures of Salts, *J. Biol. Chem.*, **34,** No. 2.

1921, The Phagocytosis of Solid Particles, I. Quartz, *J. Gen. Physiol.*, **3,** 439.

1921, The Phagocytosis of Solid Particles, II. Carbon, *J. Gen. Physiol.*, **3,** 465.

1921, Phagocytosis of Solid Particles, III. Carbon and Quartz, *J. Gen. Physiol.*, **3,** 575.

1922, Adhesiveness of Leucocytes to Solid Surfaces, *J. Gen. Physiol.*, **5,** 143.

1922, Effect of Hydrogen Ion Concentration on Phagocytosis and Adhesiveness of Leucocytes, *J. Gen. Physiol.*, **5,** 169.

1922, Phagocytosis of Solid Particles, III. Carbon and Quartz in Solutions of Varying Acidity, *J. Gen. Physiol.*, **5,** 311.

1922, Temperature Coefficient of Phagocytosis, *J. Gen. Physiol.*, **4,** 331.

1922, Theoretical Response of Living Cells to Contact with Solid Bodies, *J. Gen. Physiol.*, **4,** 373.

Fernau, W., 1914, Die Niere von Anodonta, *Z. wiss. Zoöl.*, **111,** 569.

Field, I. A., 1916, The Ultimate Sources of Marine Food, *Trans. Am. Fish. Soc.*, **45,** No. 3.

Fink, D. E., 1925, Physiological Studies on Hibernation in the Potato Beetle, *Leptinotarsa decemlineata*, *Biol. Bull.*, **49,** 381.

Fischer, M. H., 1901, Ionization in Its Physiological and Pathological Relations, *Med. Record*, Mar. 30, 1901.

1902, Further Experiments on Artificial Parthenogenesis in Annelids, *Am. J. Physiol.*, **7,** No. 3.

1903, Artificial Parthenogenesis in Nereis, *Am. J. Physiol.*, **9,** No. 2.

Fletcher, W. M., 1902, The Relation of Oxygen to the Survival Metabolism of Muscle, *J. Physiol.*, **28,** 474.

1913, Lactic Acid Formation, Survival Respiration, and Rigor Mortis in Mammalian Muscle, *J. Physiol.*, **47,** 361.

———, and G. M. Brown, 1914, Carbon Dioxide Production of Heat Rigor in Muscle, and the Theory of Intra-molecular Oxygen, *J. Physiol.*, **48,** 177.

———, and F. G. Hopkins, Respiratory Process in Muscle and the Nature of Muscular Motion, *Proc. Roy. Soc. London*, **89B,** 444.

Forbes, Alexander, 1912, Reflex Inhibition of Skeletal Muscle, *Quart. J. Exptl. Physiol.*, **5,** 149.

1912, Reflex Rhythm Induced by Concurrent Excitation and Inhibition, *Proc. Roy. Soc. London*, **85B,** 289.

1922, The Interpretation of Spinal Reflexes in Terms of Present Knowledge of Nerve Conduction, *Physiol. Rev.*, **2,** No. 3.

———, and Rappleye, 1917, Effect of Temperature Change on Rhythm in Human Electromyogram, *Am. J. Physiol.*, **42,** 228.

1917, Rate of Discharge of Central Neurones, *Proc. Nat. Acad. Sci.*, **3,** 12.

Fosse, R., 1913, The Presence of Urea in the Invertebrates and in Their Excretory Products, *Bull. sci. pharmacol.*, **20,** 644.

Fredericq, Henri, 1913, Experimental Studies on the Heart Physiology of Octopus, *Bull. acad. roy. de Belg. Cl. Sc.*, 758.

Fredericq, Leon, 1882, Sur la Regulation de la Temperature chez les animaux a sang froid, *Arch. biol.*, **3,** 687.

1885, Influence du Milieu ambiant sur la composition du sang des animaux aquatiques, *Arch. Zoöl. Exp.*, **3,** 34.

1901, Sur la concentration moleculaire du sang at des tissus chez les animaux aquatiques, *Bull. acad. roy. de Belg. Cl. Sc.* **8,** 428.

Fries, J. A., 1921, The Respiratory Quotient and Its Uncertainty, *Am. J. Physiol.*, **55,** No. 1.

Fry, Henry J., 1924, Cell Dissection by Hand, *Anat. Record*, **28,** No. 5.

Fry, H. J. B., 1909, Action of the Visceral Nerves upon the Heart in Cephalopods, *J. Physiol.*, **39,** 184.

Fry, H. K., 1913, Blood Volume and Surface Area of Cold Blooded Animals, *J. Physiol.*, **47,** 9. Also, *Quart. J. Exptl. Physiol.*, **7,** 185.

Funk, Cassimir, 1922, The Vitamins, translated by Dubin, The Williams and Wilkins Company, Baltimore.

Fürth, O. von, 1903, Vergleichende chemische Physiologie der niederen Tiere, 670 pp., Gustav Fischer, Jena.

Gage, S. H., 1917, Glycogen in the Nervous System of Vertebrates, *J. Comp. Neurol.*, **27,** No. 4.

Gaines, W. L., 1915, The Physiology of Lactation, *Am. J. Physiol.*, **38,** 285.

Gantrelet, Jean, 1910, Contributions to the Study of Organ Extracts, *C. R. Soc. Biol. Paris*, **69,** 443.

Gardner, J. A., and C. Leetham, 1914, Respiration Experiments in Fresh-water Fishes, *Biochem. J.*, **8,** 374 and 591.

Garrey, W. E., 1912, Effects of the Vagi upon the Heart Block and Ventricular Rate, *Am. J. Physiol.*, **30,** No. 6.

1915, Resistance of Fresh-water Fish to Changes in Osmotic and Chemical Conditions, *Am. J. Physiol.*, **39,** 313.

1915, Some Cryoscopic and Osmotic Data, *Biol. Bull.*, **28,** No. 2.

1920, The Action of Inhibitory Nerves on Carbon Dioxide Production in the Heart Ganglion of *Limulus*, *J. Gen. Physiol.*, **3,** 163.

1920, The Temperature Coefficient of Carbon Dioxide Production by the Heart Ganglion of *Limulus polyphemus*, *J. Gen. Physiol.*, **3,** 49.

1920, The Temperature Coefficient of the Neurogenic Rhythm of the Heart of *Limulus polyphemus*, *J. Gen. Physiol.*, **3,** 41.

———, and A. R. Moore, 1915, Peristalsis and Coordination in the Earthworm, *Am. J. Physiol.*, **39,** No. 2.

Gaskell, J. F., 1912, Supra-renal Gland of Petromyzon, *J. Physiol.*, **44,** 59.

1914, The Chromaffine System of Annelids and the Relation of This System to the Contractile Vascular System in the Leech, *Phil. Trans. Roy. Soc. London*, **205B,** 153.

1919, Adrenaline in Annelids, *J. Gen. Physiol.*, **2,** 173.

Gaskell, W. H., 1882, *Croonian Lecture*—On the Rhythm of the Heart of the Frog, and of the Nature of the Action of the Vagus Nerve, *Phil. Trans. Roy. Soc. London*, 993.

1884, On the Augmentor (Accelerator) Nerves of the Heart of Cold-blooded Animals, *J. Physiol.*, **5,** 46.

1886, On the Structure, Distribution, and Functions of the Nerves which Innervate the Visceral and Vascular Systems, *J. Physiol.*, **7,** 1.

1916, The Involuntary Nervous System, Longmans, Green & Co., London.

Gault, C. C., 1917, Physiology of the Atrio-ventricular Connection in the Turtle—The Influence of the Vagi and of the Sympathetic Nerves on Its Rhythm Forming Power, *Am. J. Physiol.*, **43,** 22.

———, and F. H. Scott, 1918, Some Points in the Nervous Regulation of Respiration in the Cat, *Am. J. Physiol.*, **45,** 554.

Gerould, J. H., 1924, Periodic Reversal of Heart Beat in a Chrysalis, *Science,* N. S., **60.**

Gesell, Robert, 1913, On the Relation of the Pulse Pressure to Renal Secretion, *Am. J. Physiol.*, **32,** No. 1.

1916, Initial Length—Initial Tension and Tone of Auricular Muscle in Relation to Myo- and Cardio-dynamics, *Am. J. Physiol.*, **39,** No. 3.

Goldschmidt, S., and A. B. Dayton, 1919, The Mechanism of Absorption from the Intestine, I. The Colon—A Contribution to the One-sided Permeability of the Intestinal Wall to Chlorides, *Am. J. Physiol.*, **48,** 419.

1919, II. On the Passage of Fluid in Two Directions through the Intestinal Wall, *Am. J. Physiol.*, **48,** 433.

1919, III. Osmotic Pressure Equilibrium between the Intestinal Content and the Blood, *Am. J. Physiol.*, **48,** 440.

1919, IV. The Behavior of Sodium and Magnesium Solutions, *Am. J. Physiol.*, **48,** 450.

1919, V. The Effect of Sodium Sulphate upon the Absorption of Sodium Chloride When the Salts Are Introduced Simultaneously into the Intestine, *Am. J. Physiol.*, **48,** 459.

1919, VI. The Influence of Calcium Salts upon the Absorption of Sodium Chloride in the Intestine, *Am. J. Physiol.*, **48,** 473.

Gomez, L., and F. H. Pike, 1909, The Histological Changes in Nerve Cells Due to Fatal Temporary Anaemia of the Central Nervous System, *J. Exptl. Med.*, **11,** No. 2.

Grave, Caswell, 1916, The Processes of Feeding in the Oyster, *Science*, **44,** 178.

———, and Frances O. Schmitt, 1925, A Mechanism for the Coordination and Regulation of the Movement of Cilia of Epithelia, *Science*, N. S., **60,** 246. *J. Morph. Physiol.*, **40,** No. 3.

Gray, J., 1923, The Mechanism of Ciliary Movement, III. Effect of Temperature, *Proc. Roy. Soc. London*, **95B.**

Green, C. W., 1898, External Stimulus and Nerve Impulse, *Am. J. Physiol.*, **1,** 104.

1898, On the Relation of the Inorganic Salts of Blood to the Automatic Activity of a Strip of Ventricular Muscle, *Am. J. Physiol.*, **2,** No. 1.

1899, Relation of Blood Salts to Heart Beat, *Am. J. Physiol.*, **3,** 82.

Greenwood, M., 1886, On Digestive Processes in Some Rhizopods, *J. Physiol.*, **7,** 253.

Griffeths, A. B., 1897, Respiratory Proteids, L. Reeve and Co., London.

Gruber, C. M., 1914, The Relation of Adrenaline to Curare and Fatigue in Normal and Denervated Muscles, *Am. J. Physiol.*, **34,** 89.

———, and C. Markel, 1918, Tonus Waves of the Sino-auricular Muscle Preparation of the Terrapin as Affected by Adrenalin, *J. Pharmacol. Exptl. Therap.* **12,** No. 1.

Gudernatsch, J. F., 1912, Treatment of Tadpoles with Thyroid and Thymus Extracts, *Anat. Record*, **11,** 357.

Haas, A. R., 1916, A Simple and Rapid Method of Studying Respiration by the Detection of Exceedingly Minute Quantities of Carbon Dioxide, *Science*, N. S., **44,** 105.

1916, The Acidity of Plant Cells as Shown by Natural Indicators, *J. Biol. Chem.*, **27,** No. 1.

1916, The Effect of the Addition of Alkali to Sea Water upon Hydrogen Ion Concentration, *J. Biol. Chem.*, **26,** No. 2.

1917, Reaction of Plant Protoplasm, *Bot. Gaz.*, **63,** No. 3.

Hackh, Ingo W. D., 1919, Bio-elements: The Chemical Elements of Living Matter, *J. Gen. Physiol.*, **1,** 429.

Haldane, J. S., 1892, A New Form of Apparatus for Measuring the Respiratory Exchange of Animals, *J. Physiol.*, **18,** 419.

1917, Organism and Environment: Breathing, Yale University Press.

1918, The Extension of the Gas Laws to Liquids and Solids, *Biochem. J.* **12,** 464.

Halliburton, W. D., 1885, The Blood of the Decapod Crustacea, *J. Physiol.*, **6,** 300.

Hamburger, H. J., 1910, The Influence of Small Amounts of Calcium on the Motion of Phagocytes, *Proc. Konink. Akad. Amsterdam,* 66.

1911, Osmotic Pressure, *Science,* N. S., **34,** 583.

Hamill, J. M., 1906, On the Mechanism of Protection of Intestinal Worms and Its Bearing on the Relation of Enterokinase to Trypsin, *J. Physiol.*, **33,** 476.

Hammar, A. G., 1908, On the Nervous System of the Larva of *Cordyalis cornuta, Ann. Ent. Soc. Am.*, **1,** No. 2, 105.

Hammett, F. S., 1924, Studies on the Thyroid Apparatus, *J. Comp. Neurol.*, **37,** No. 1.

Hanström, B., 1925, The Olfactory Centers in Crustaceans, *J. Comp. Neurol.*, **38,** No. 3.

Hargitt, C. W., 1923, The Digestive System of the Periodical *Cicada, Biol. Bull.*, **45,** 200.

Harkins, W. H., and E. B. Keith, 1924, The Oriented Wedge Theory of Emulsions and the Inversion of Emulsions, *Science,* N. S., **49,** 463.

Harvey, E. N., 1914, Is the Fertilization Membrane of *Arbacia* Eggs a Precipitation Membrane? *Biol. Bull.*, **27,** No. 5.

Harvey, William, 1628 (reprint 1894), An Anatomical Dissertation upon the Movement of the Heart and Blood in Animals.

Hatschek, E., 1919, An Introduction to the Physics and Chemistry of Colloids, 3d. ed., P. Blakiston's Son and Company, Philadelphia.

Hawk, P. B., 1923, Practical Physiological Chemistry, 8th ed., P. Blakiston's Son and Company Philadelphia.

Heath, H., 1905, The Excretory and Circulatory Systems of *Cryptochiton stelleri, Biol. Bull.*, **9,** No. 4.

1924, The External Development of Certain Phyllopods, *J. Morphology,* **38,** No. 4.

Hecht, S., 1918, The Physiology of *Ascidia atra* Lesuear, *J. Exptl. Zoöl.*, **25.**

1919, Analysis of the Relation between the Temperature and Duration of a Biological Process, *Proc. Nat. Acad. Sci.*, **5,** 146.

Heilbrunn, L. V., 1915, Physical Change in the Egg of Arbacia, *Biol. Bull.*, **29,** No. 3.

1915, The Measurement of Oxidation in the Sea Urchin Egg, *Science,* N. S., **42,** 615.

1920, Studies in Artificial Parthenogenesis, III. Cortical Change and the Initiation of Maturation in the Egg of *Cumingea, Biol. Bull.*, **38,** No. 5.

1920, The Physical Conditions Which Determine the Appearance of the Spindle in the Sea Urchin Eggs, *J. Exptl. Zoöl.*, **30,** No. 2.

1920, The Physical Effects of Anesthetics upon Living Protoplasm, *Biol. Bull.*, **39,** No. 6.

1924, The Colloid Chemistry of Protoplasm, *Am. J. Physiol.*, **69,** No. 1.

1924, The Colloid Chemistry of Protoplasm, III. The Viscosity of Protoplasm at Various Temperatures, *Am. J. Physiol.*, **68,** No. 3.

1924, The Surface Tension Theory of Membrane Elevation, *Biol. Bull.*, **46,** No. 6.

1925, Colloidal Change and Mitosis, *Arch. Mikr. Anat. u. Entwickl. Mech.* **104,** 313.

Henderson, L. J., 1913, The Fitness of the Environment, The Macmillan Company, New York.

1916, Volume in Biology, *Proc. Nat. Acad. Sci.*, **2,** 654.

Henderson, Y., and H. W. Haggard, 1918, Respiratory Regulation of the Carbon Dioxide Capacity of the Blood, *J. Biol. Chem.*, **33,** 333, 345, 355.

Henri, V., 1906, Perivisceral Fluid of Sea Urchin, *C. R. Soc. Biol. Paris*, **60,** 880.

Henze, M., 1910, Influence of Oxygen Pressure on the Gas Metabolism of Some Marine Animals, *Biochem. Z.*, **26,** 255, abstract in *Chem. Abstracts*, **4,** 3104.

1911, Examination of the Blood of *Ascidia*, *Z. physiol. Chem.*, **72,** 494, abstract in *Chem. Abstracts*, **5,** 3293.

Herwerden, M. van, 1909, Gastric Digestion in Fish, *Z. physiol. Chem.*, **56,** 53, abstract in *Chem. Abstracts*, **3,** 1420.

Hess, W. N., 1918, A Seasonal Study of the Kidney of the Five-spined Stickelback *Eucalia*, *Anat. Record*, **14,** No. 2.

1925, Nervous System of the Earthworm, *Lumbricus terrestris*, *J. Morph. Physiol.*, **40,** No. 2.

1925, Photo-receptors of *Lumbricus terrestris*, with Special Reference to Their Distribution, Structure, and Function, *J. Morph. Physiol.*, **41,** No. 1.

Hickernell, L. M., 1923, The Digestive System of the Periodical *Cicada*, *Tibicen septendecim*, *Biol. Bull.*, **45,** 213.

Hill, A. V., 1911, A New Form of Differential Micro-calorimeter for the Estimation of Heat Production in Physiological, Bacteriological, or Ferment Actions, *J. Physiol.*, **43,** 261.

1911, The Position Occupied by the Production of Heat in the Chain of Processes Constituting a Muscular Contraction, *J. Physiol.*, **42,** 1.

1911, The Total Energy Exchanges of Intact Cold-blooded Animals at Rest, *J. Physiol.*, **43,** 379.

1912, The Absence of Temperature Changes during the Transmission of a Nervous Impulse, *J. Physiol.;* **43,** 433.

1912, The Delayed Heat Production of Muscles Stimulated in Oxygen, *J. Physiol.*, **45,** 35.

1912, The Heat Production of Surviving Amphibian Muscles, During Rest, Activity, and Rigor, *J. Physiol.*, **44,** 466.

1913, The Absolute Mechanical Efficiency of the Contraction of an Isolated Muscle, *J. Physiol.*, **46,** 435.

1913, The Energy Degraded in the Recovery Processes of Stimulated Muscles, *J. Physiol.*, **46,** 28.

1913, The Heat Production in Prolonged Contractions of an Isolated Frog's Muscle, *J. Physiol.*, **47,** 305.

1914, The Oxidative Removal of Lactic Acid, *J. Physiol.*, **48,** 10.

1924, Muscular Activity and Carbohydrate Metabolism, *Science*, N. S., **60,** No. 1562.

——— and A. M., 1913, Calorimetry Experiments on Warm-blooded Animals, *J. Physiol.*, **46,** 81.

——— ———, and W. Hartree, 1920, The Four Phases of Heat Production of Muscle, *J. Physiol.*, **54,** 84.

Hilton, W. A., 1911, Some Remarks on the Motor and Sensory Tracts of Insects, *J. Comp. Neurol.*, **21,** 383.

1917, The Central Nervous System of Simple Crustacea, *J. Comp. Neurol.*, **28,** No. 2.

Hitchcock, D. I., 1924, Proteins and the Donnan Equilibrium, *Physiol. Rev.*

Hoeber, R., 1914, Physikalische Chemie der Zelle und der Gewebe, 4 Auf., Engelmann, Leipzig.

Hogben, L. T., and F. R. Winton, 1924, Introduction to Recent Advances in Comparative Physiology, The Macmillan Company, New York.

Hollande, A. Ch., 1911, Etude Histologique comparee du sang des Insectes a Hemorrhee et du Insectes sans hemorrhee, *Arch. Zoöl. Exp.* (5), **6,** 283.

1915, On the Physiology of the Pericardial Cells of Insects, *Arch. Zoöl. Exp.*, **55,** 67.

HOLMES, S. J., 1914, The Life of Isolated Larval Muscle Cells, *Science*, N. S., **40,** No. 1025.

HOOKER, D. R., 1920, The Functional Activity of the Capillaries and Venules, *Am. J. Physiol.*, **54,** No. 1.

1921, Evidence of Functional Activity on the Part of the Capillaries and Venules, *Physiol. Rev.*, **1,** 112.

HOSKINS, E. R. and M. M., 1919, Growth and Development of Amphibia as Affected by Thyroidectomy, *J. Exptl. Zoöl.*, **29,** 1.

———1920, Inter-relation between Thyroid and Hypophysis in Growth and Development of Frogs, *Endocrinology*, **4,** 1.

HOSKINS, R. G., 1912, The Adrenal Glands, *Cleveland Med. J.*, **11,** 180.

———, R. E. L. GUNNING, and E. L. BERRY, 1916, The Effects of Adrenin on the Distribution of the Blood, I. Volume Changes and Venous Discharge in the Limb, *Am. J. Physiol.*, **41,** No. 4.

HOWELL, W. H., 1910, Chemical Regulation of Body Processes by Means of Activators, Kinases, and Hormones, *Science*, N. S., **31,** 93.

1916–1917, The Coagulation of the Blood, *Harvey Lectures.*

1924, Textbook of Physiology, 9th ed., W. B. Saunders Company, Philadelphia.

HOWLAND, R. B., 1923, Application of the Murexide Test to *Amoeba verrucosa* and *Paramoecium caudatum*, *Proc. Soc. Exptl. Biol. Med.*, **20,** 469.

1923, Notes on the Dissection of *Amoeba verrucosa*, *Proc. Soc. Exptl. Biol. Med.*, **20,** 471.

1923, Studies on the Contractile Vacuoles of *Amoeba verrucosa* and *Paramoecium caudatum*, *Proc. Soc. Exptl. Biol. Med.*, **20,** 470.

1924, Dissection of the Pellicle of *Amoeba verrucosa*, *J. Exptl. Zoöl.* **40,** No. 2.

1924, *Experiments on the Contractile Vacuole of Amoeba verrucosa and Paramoecium caudatum*, *J. Exptl. Zoöl.*, **40,** No. 2.

1924, On Excretion of Nitrogenous Waste as a Function of the Contractile Vacuole *J. Exptl. Zoöl.*, **40,** No. 2.

HUNT, R., and D. W. HARRINGTON, 1897, Note on the Physiology of the Cardiac Nerves of the Calf, *J. Exptl. Med.*, **2,** No. 6.

1897, Notes on the Physiology of the Cardiac Nerves of the Opossum, *J. Exptl. Med.*, **2,** No. 6.

HYDE, IDA H., 1902, The Nervous System of *Gonionema murbachii*, *Biol. Bull.*, **4,** No. 1.

1906, A Reflex Respiratory Center, *Am. J. Physiol.*, **16,** 368.

HYMAN, L. H., 1918, Carbon-dioxide Production in Relation to Regeneration in *Planaria*, *Am. J. Physiol.*, **48,** 340.

1918, Suggestions Regarding the Causes of Bio-electric Phenomena, *Science*, N. S., **48,** 918.

1919, Oxygen Consumption in *Planaria*, *Biol. Bull.*, **37.**

1919, Physiological Studies on *Planaria*, I. Oxygen Consumption in Relation to Feeding and Starvation, *Am. J. Physiol.*, **49,** 377.

1919, Physiological Studies on *Planaria*, II. Oxygen Consumption in Relation to Regeneration, *Am. J. Physiol.*, **50,** 67.

1919, The Rate of Respiratory Metabolism in *Planaria*, *Am. J. Physiol.*, **49,** 420.

———, B. H. WILLIER, and S. A. REFENBURG, 1924, A Respiratory and Histochemical Investigation of the Source of the Increased Metabolism after Feeding, *J. Exptl. Zoöl.*, **40,** No. 3.

IRWIN, MARIAN, 1918, The Nature of Sensory Stimulation by Salts, *Am. J. Physiol.*, **47,** 265.

JACOBS, M. H., 1919, Acclimatization as a Factor Affecting the Upper Thermal Death Points of Organisms, *J. Exptl. Zoöl.*, **27,** 427.

1920, A Simple Method of Measuring the Rate of Respiration of Small Animals, *Am. Naturalist*, **54,** 91.

1920, Production of Intra-cellular Acidity by Neutral and Alkaline Solutions Containing Carbon dioxide, *Am. J. Physiol.*, **53.**

1922, Effects of Carbon-dioxide on the Consistency of Protoplasm, *Biol. Bull.*, **41.**

Jenkins, O. P., and A. J. Carlson, 1903, The Rate of Nervous Impulse in Certain Molluscs, *Am. J. Physiol.*, **8,** 251.

Jerusalem, E., and E. H. Starling, 1910, Significance of Carbon-dioxide for the Heart, *J. Physiol.*, **40,** 279.

Joel, A., 1919, Importance of Temperature on Oxygen Consumption of Poikilothermous Animals, *Z. Physiol. Chem.*, **107,** 231, abstract in *Chem. Abstracts*, **14,** 2371.

Johnson, G. E., 1924, Giant Fibers in Crustaceans, with Special Reference to *Cambarus* and *Palaemonetes*, *J. Comp. Neurol.*, **36,** No. 4.

Jona, J. L., 1912, Cryscopic Determination of the Osmotic Pressure of the Blood and Body Fluids of Some Australian Animals, *Biochem. J.*, **6,** 130.

Jordan, H. E., 1914, The Microscopic Structure of Mammalian Cardiac Muscle, with Special Reference to So-called Muscle Cells, *Anat. Record*, **8,** No. 9.

1916, The Microscopic Structure of the Leg Muscle of the Sea Spider, *Anoplodactylus lentus*, *Anat. Record*, **10,** No. 7.

1917, Cardiac and Skeletal Muscle of *Scorpio*, *Anat. Record*, **13,** 1.

1917, Striped Cardiac and Skeletal Muscle of *Limulus*, *Carnegie Inst. Pub., Wash.*, No. 251, 273.

———, and James Bardin, 1913, The Relation of the Intercalated Discs to the So-called Segmentation and Fragmentation of Heart Muscle, *Anat. Anz.*, **43,** Nos. 23 and 24.

———, and J. B. Banks, 1917, A Study of the Intercalated Discs of the Heart of the Beef, *Am. J. Anatomy*, **22,** No. 2.

Joseph, D. R., and S. J. Meltzer, 1911, Action of Sodium and Calcium upon the Irritability of the Muscles of the Frog, *Am. J. Physiol.*, **29,** 1.

Just, E. E., 1915, An Experimental Analysis of Fertilization in *Platynereis megalops*, *Biol. Bull.*, **28,** No. 2.

1915, The Morphology of Normal Fertilization in *Platynereis megalops*, *J. Morphology*, **26,** No. 2.

1919, Fertilization Reaction in *Echinarachnius parma*, *Biol. Bull.*, **36,** No. 1.

1919, The Cortical Response of the Egg to Insemination, *Biol. Bull.*, **36,** No. 1.

1919, The Nature of the Activation of the Egg by Butyric Acid, *Biol. Bull.*, **36,** No. 1.

1919, The Rôle of Fertilizin in Straight and Cross Fertilization, *Biol. Bull.*, **36,** No. 1.

1920, The Fertilization Reaction in *Echinarachnius parma*, *Biol. Bull.*, **39,** No. 5.

1922, Initiation of Development in the Egg of *Arbacia*, *Biol. Bull.*, **43,** No. 6.

1922, Initiation of Development in the Egg of *Arbacia*, II. Fertilization of Eggs in Various Stages of Artificially Induced Mitosis, *Biol. Bull.*, **43,** No. 6.

1922, Initiation of Development in the Egg of *Arbacia*, III. The Effect of *Arbacia* Blood on the Fertilization Reaction, *Biol. Bull.*, **43,** No. 6.

1922, The Effect of Sperm Boiled in Oxalated Sea Water in Initiating Development, *Science*, N. S., **56,** 202.

1922, The Fertilization Reaction in *Echinarachnius parma*, V. The Existence in the Inseminated Egg of a Period of Special Susceptibility to Hypotonic Sea Water, *Am. J. Physiol.*, **61,** No. 3.

1923, The Fertilization Reaction in *Echinarachnius parma*, VI. The Necessity of the Egg Cortex for Fertilization, *Biol. Bull.*, **44,** No. 1.

1923, The Fertilization Reaction in *Echinarachnius parma*, VII. The Inhibitory Action of Blood, *Biol. Bull.*, **44,** No. 1.

Kanitz, A., 1907, Influence of Temperature on Contractile Vacuole of Infusoria, *Biol. Cent.*, **27,** 11.

1915, Temperatur und Lebensvorgänge, Gebruder Borntraeger, Berlin.

Kappers, C. U., and Ae B. Droogleeven Fortuyn, 1920, Vergleichende Anatomie des Nervensystems, DeE. F. Bohn, Haarlem.

Kellicott, W. E., 1916, The Effects of Low Temperature upon the Development of *Fundulus*, *Am. J. Anatomy*, **20,** No. 3.

Kellogg, J. L., 1916, Opinions on Some Ciliary Activities, *Science*, N. S., **44,** 852.

Kendall, E. C., 1918, The Isolation and Identification of the Thyroid Hormone, *Am. J. Physiol.*, **45,** 540.

Kennaway, E. L., 1920, Notes on the Evolution of Proteins, *Chem. News* **120,** 13.

Kite, G. L., 1913, The Physical Properties of the Protoplasm of Certain Animal and Plant Cells, *Am. J. Physiol.*, **32,** No. 2.

1914, Some Structural Transformations of the Blood Cells of Vertebrates, *Proc. Soc. Exptl. Biol. Med.*, **11,** No. 4.

Knowlton, F. P., and A. R. Moore, 1917, Note of the Reversal of Reciprocal Inhibition in the Earthworm, *Am. J. Physiol.*, **44,** 490.

Koch, W., 1917, Der Herzschlag von *Anodonta* unter Natürlischen und Kunstlichen Bedingungen, *Pflüger's Arch. ges. Physiol.*, **166,** 281.

Kossel, A., and A. Mathews, 1898, Zur Kenntniss der Trypsinwirkung, *Hoppe-Seyler's Z. physiol. Chem.*, **25,** Nos. 3 and 4.

Krogh, A., 1916, The Respiratory Exchange of Animals and Man, Longmans, Green & Co., London.

1919, Rate of Diffusion of Gases Through Animal Tissues, *J. Physiol.*, **52,** 391.

1919, The Number and Distribution of the Capillaries in Muscles with the Calculations of the Oxygen Pressure Necessary for Supplying the Tissue, *J. Physiol.*, **52,** 457.

1919, The Supply of Oxygen to the Tissues and the Regulation of the Capillary Circulation, *J. Physiol.*, **52,** 405.

1922, The Anatomy and Physiology of Capillaries, Yale University Press.

———, and Leitch, 1919, Respiratory Function of Blood in Fishes, *J. Physiol.*, **52,** 288.

Krummacher, O., 1918, Studies on the Evolution of Heat by Ascaris, A Contribution to the Study of Life without Oxygen, *Z. Biol.*, **69,** 293.

Kugler, K., 1918, The Digestion and Absorption of Protein, *Z. Biol.*, **69,** 437.

Langley, J. N., 1916, Autonomic Nervous System, *J. Physiol.*, **50,** 225.

Lankester, Sir E. Ray, 1886, On Green Oysters, *Quart. J. Mic. Sci.*, **26,** 71.

1893, Phagocytes of Green Oysters, *Nature*, **48,** 75.

Larson, J. A., 1919, Functional Correlation of Hypophysis and Thyroid, *Am. J. Physiol.*, **49,** 55.

Latham, P. W., 1908, Synthesis of Living Albumen, *Biochem. J.*, **3,** 241.

Latimer, Caroline, 1898, On the Modification of Rigor Mortis Resulting from Previous Fatigue of the Muscle in Cold-blooded Animals, *Am. J. Physiol.*, **2,** No. 1.

Laurens, Henry, 1914, The Influence of Temperature on the Rate of the Heart Beat in Amblystoma Embryos, *Am. J. Physiol.*, **35,** No. 2.

1916, The Physiology of the Atrio-ventricular Connection in the Turtle, I. Disturbances of A.V. Conduction, *Am. J. Physiol.*, **42,** 89.

1916, The Physiology of the Atrio-ventricular Connection in the Turtle, *Am. J. Physiol.*, **42,** 513.

1917, Irritability and Rhythm Formation, *Am. J. Physiol.*, **42,** No. 4.

Lee, Milton O., 1925, On the Mechanism of Respiration in Certain Orthoptera, *J. Exptl. Zoöl.*, **41,** No. 2.

Legendre, R., 1907, Variations de Densite, de Temperature, et de Teneur en Oxygene de l'eau de la Cote, a Concarneau, *C. R. Soc. Biol.*, **63,** 611.

Leitch, I., 1916, The Function of Haemoglobin in Invertebrates with Special Reference to Planorbis and Chironomous Larvae, *J. Physiol.*, **50.**

1916, The Function of the Heart in Invertebrates, *J. Physiol.*, **50,** 372.

Lesser, E. J., 1908, Chemische Prozesse bei Regenwürmer, *Z. Biol.*, **50,** 421.

———, and E. W. Taschenberg, 1908, Ferments of Ring Worms, *Z. Biol.*, **50,** 446.

Lewis, G. N., and M. Randall, 1923, Thermodynamics, McGraw-Hill Book Company, Inc., New York.

Lewis, H. B., and Minna E. Jewell, 1916, The Occurrence of Lichenase in the Digestive Tract of Invertebrates, *Proc. Soc. Exptl. Biol. Med.*, **14,** 59.

Lewis, M. L., 1916, Advantages of Sea Water as a Medium, *Anat. Record*, **10,** 287.

Lewis, Thomas, 1916, The Speed of the Excitatory Process in the Vertebrate Heart, Electro-cardiogram Studies on Toad, Tortoise, Dog, Man, Bird, *Phil. Trans. Roy. Soc. London*, **207B,** 221.

1917, The Motion of the Mammalian Heart, *Proc. Roy. Soc. London*, **89B,** 560.

Liddell, H. S., 1925, The Growth of the Head in Thyroidectomized Sheep, *Anat. Record*, **30,** No. 5.

Lillie, F. R., 1913, The Behavior of the Spermatozoa of *Nereis* and *Arbacia* with Special Reference to Egg-extractives, *J. Exptl. Zoöl.*, **14,** No. 4.

1919, Problems of Fertilization, University of Chicago Press.

1921, On the Measure of Specificity in Fertilization between Two Associated Species of the Sea Urchin, Genus *Strongylocentrotus*, *Biol. Bull.*, **40,** No. 1.

1921, On the Question of Superposition of Fertilization on Parthenogenesis in *Strongylocentrotus purpuratus*, *Biol. Bull.*, **40,** No. 1.

Lillie, R. S., 1909, The General Biological Significance of Changes in Permeability of the Surface Layer of Living Cells, *Biol. Bull.*, **17,** 188.

1909, The Relation of Ions to Contractile Processes, IV. The Influence of Various Electrolytes in Restoring Muscular Activity After Its Loss in Solutions of Sugar and of Magnesium Chloride, *Am. J. Physiol.*, **24,** No. 5.

1910, The Physiology of Cell Division—The Action of Isotonic Solutions of Neutral Salts on Fertilized Eggs of *Asterias* and *Arbacia*, *Am. J. Physiol.*, **26,** No. 1.

1911, The Relation of Stimulation and Conduction in Irritable Tissues to Changes in the Permeability of the Limiting Membrane, *Am. J. Physiol.*, **28,** 197.

1912, Antagonism Between Salts and Anaesthetics, I. On the Conditions of the Anti-stimulating Action of Anaesthetics with Observations on Their Protective or Antitoxic Action, *Am. J. Physiol.*, **29,** 372.

1912, II. Decrease by Anesthetics in the Rate of Toxic Action of Pure Isotonic Salt Solution on Unfertilized Starfish and Sea Urchin Eggs, *Am. J. Physiol.*, **30,** 1.

1913, The Formation of Indophenol Blue at the Nuclear and Plasma Membranes of Frog's Blood Corpuscles and Its Acceleration by Induction Shocks, *J. Biol. Chem.*, **15,** 237.

1913, The Rôle of Membranes in Cell Processes, *Pop. Sci. Monthly*, February, 1913.

1914, The General Physico-chemical Conditions of Stimulation in Living Organisms, *Pop. Sci. Monthly*, June, 1914.

1916, Electrolytic Local Action as the Basis of Propagation of the Excitation Wave, *Am. J. Physiol.*, **41,** 126.

1916, Mass Action in the Activation of Unfertilized Starfish Eggs by Butyric Acid, *J. Biol. Chem.*, **24,** No. 3.

1917, The Conditions Determining the Rate of Entrance of Water into Fertilized and Unfertilized Eggs of Arbacia, and the General Relation of Changes of Permeability to Activation, *Am. J. Physiol.*, **43,** 43.

1920, The Nature of Protoplasmic and Nervous Transmission, *J. Phys. Chem.*, **24,** 165.

1923, Protoplasmic Action and Nervous Action, University of Chicago Press.

Lindemann, W., 1914, Zur Lehre von der Funcktionen der Niere, *Ergebnisse Physiol.*, **14,** 618.

Lingle, D. J., 1902, The Importance of Sodium Chloride in Heart Activity, *Am. J. Physiol.*, **8,** No. 2.

1910, The Mechanism of Tone in Plain Muscle, *Am. J. Physiol.*, **26,** 361.

Linn, R. K. S., 1918, Respiratory Mechanism of Shore Crab, *Proc. Roy. Soc. Edinburgh*, **38,** 28.

Linstedt, Ph., 1916, Respiration and Metabolism of Cold-blooded Animals, *Z. Fisch.*, **14,** 193, abstract in *Chem. Abstracts*, **10,** 1765.

Lipschutz, Alexander, 1918, Nutrition of Aquatic Animals, *Biol. Zentr.*, **38,** 196.

Lloyd, Dorothy Jordan, 1913, A Critical Analysis of Delage's Method of Producing Artificial Parthenogenesis in the Eggs of the Sea Urchin, *Arch. Entwickl. Mech. Organ.*, **38,** H. 3.

Loeb, Jacques, 1899, Nature of the Process of Fertilization, *Biological Lectures*, Marine Biological Laboratory, Woods Hole.

1900, Comparative Physiology of the Brain and Comparative Psychology, G. P. Putnam's Sons, New York.

1900, Further Experiments on Artificial Parthenogenesis and the Nature of the Process of Fertilization, *Am. J. Physiol.*, **4,**, No. 4.

1900, On the Nature of the Process of Fertilization and the Artificial Production of Normal Larvae (Plutei) from the Unfertilized Eggs of the Sea Urchin, *Am. J. Physiol.*, **3,** 135.

1901, Experiments on Artificial Parthenogenesis in Annelids (*Chaetopterus*) and the Nature of the Process of Fertilization, *Am. J. Physiol.*, **4,** No. 9.

1903, Artificial Parthenogenesis in Molluscs, *Univ. Calif. Pub. Phys.*, **1,** No. 3.

1903, On a Method by Which the Eggs of a Sea Urchin (*Strongylocentrotus*) Can Be Fertilized with the Sperm of a Starfish (*Asterias ochracea*), *Univ. Calif. Pub. Phys.*, **1,** 1.

1903, The Fertilization of the Egg of the Sea Urchin by the Sperm of the Starfish, *Univ. Calif. Pub. Phys.*, **1,** No. 6.

1904, On the Influence of the Reaction of the Seawater on the Regeneration and Growth of Tubularians, *Univ. Calif. Pub. Phys.*, **1,** No. 15.

1905, Artificial Membrane Formation and Chemical Fertilization in a Starfish (*Asterina*), *Univ. Calif. Pub. Phys.*, **2.**

1905, On an Improved Method of Artificial Parthenogenesis, *Univ. Calif. Pub. Phys.*, **2,** No. 14.

1905, On Chemical Methods by Which the Eggs of a Mollusc Can Be Caused to Become Mature, *Univ. Calif. Pub. Phys.*, **3,** No. 1.

1905, Studies in General Physiology, *Decennial Pub.*, University of Chicago Press.

1906, The Stimulating and Inhibitory Effects of Magnesium and Calcium upon the Rhythmical Contractions of a Jellyfish (*Polyorchis*), *J. Biol. Chem.*, **1,** No. 6.

1906, The Toxicity of Atmospheric Oxygen for the Eggs of the Sea Urchin after the Process of Membrane Formation, *Univ. Calif. Pub. Phys.*, **3,** No. 3.

1907, The Chemical Character of the Process of Fertilization, and Its Bearing upon the Theory of Life Phenomena, *Univ. Calif. Pub. Phys.*, **3,** No. 10.

1910, Ueber den Autokatalytischen Character der Kernsynthese bei der Entwicklung, *Biol. Cent.*, **30,** No. 10

1911, How Does the Act of Fertilization Save the Life of the Egg? *Harvey Lectures.*
1911, The Rôle of Salts in the Preservation of Life, *Science,* N. S., **34,** No. 881.
1912, The Comparative Efficiency of Weak and Strong Bases in Artificial Parthenogenesis, *J. Exptl. Zoöl.,* **13,** No. 4.
1912, The Mechanistic Conception of Life, University of Chicago Press.
1913, Artificial Parthenogenesis and Fertilization, University of Chicago Press.
1913, Avogadro's Law and the Absorption of Water by Animal Tissues in Crystalloid and Colloid Solutions, *Science,* N. S., **37,** 427.
1914, Artificial Parthenogenesis and Fertilization, *Science,* N. S. **39,** 908.
1914, Cluster Formation of Spermatozoa Caused by Specific Substances from Eggs, *J. Exptl. Zoöl.,* **17,** No. 1.
1914, On Some Non-specific Factors for the Entrance of the Spermatozoon into the Egg, *Science,* N. S., **40,** 316.
1915, On the Nature of the Conditions Which Determine or Prevent the Entrance of the Spermatozoon into the Egg, *Am. Naturalist,* **49,** 257.
1915, The Mechanism of Antagonistic Salt Action, *Proc. Nat. Acad. Sci.,* **1,** 473.
1915, The Stimulation of Growth, *Science,* N. S. **41,** No. 1063.
1916, The Mechanism of the Diffusion of Electrolytes Through the Membranes of Living Cells, *J. Biol. Chem.,* **27.**
1916, The Sex of Parthenogenetic Frogs, *Proc. Nat. Acad. Sci.,* **2.**
1916, The Temperature Coefficient for the Duration of Life, *Proc. Nat. Acad. Sci.,* **2,** 456.
1917, The Similarity of the Action of Salts upon the Swelling of Animal Membranes and of Powdered Colloids, *J. Biol. Chem.,* **31,** No. 2.
1918, Diffusion of Electrolytes Through the Membranes of Living Cells, V. The Additive Effect of Salt and Base and the Antagonistic Effect of Salt and Acid, *J. Biol. Chem.,* **32.**
1918, Ionization of Proteins and Antagonistic Salt Action, *J. Biol. Chem.,* **33,** No. 3.
1918, Physiologically Balanced Salt Solutions, *J. Biol. Chem.,* **34,** 503.
1918, The Influence of Electrolytes upon the Osmotic Pressure of Gelatine Solutions, *J. Biol. Chem.,* **35.**
1918, The Influence of Neutral Salts upon the Viscosity of Gelatine Solutions, *J. Biol. Chem.,* **34,** No. 2.
1918, The Stoichiometrical Character of the Action of Neutral Salts upon the Swelling of Gelatine, *J. Biol. Chem.,* **34,** No. 1.
1918, Volumetric Analysis of Ion-protein Compounds; the Significance of the Isoelectric Point for the Purification of Amphoteric Colloids, *J. Gen. Physiol.,* **1,** 237.
1919, The Influence of the Valency of Anions upon the Physical Properties of Gelatine, *J. Gen. Physiol.,* **1,** 559.
1920, Ion Series and the Physical Properties of Proteins, *J. Gen. Physiol.,* **3,** No. 2.
1920, On the Cause of the Influence of Ions on the Rate of Diffusion of Water Through Collodion Membranes, *J. Gen. Physiol.,* **2,** No. 4.
1920, The Proteins and Colloid Chemistry, *Science,* N. S., **52,** No. 1350.
1921, Ion Series and the Physical Properties of Proteins, III. The Action of Salts in Low Concentration, *J. Gen. Physiol.,* **3,** No. 3.
1921, Chemical and Physical Behavior of Casein Solutions, *J. Gen. Physiol.,* **3,** No. 4.
1921, Donnan Equilibrium and the Physical Properties of Proteins, *J. Gen. Physiol.,* **3,** No. 5.
1921, Donnan Equilibrium and the Physical Properties of Proteins, I. Membrane Potentials, *J. Gen. Physiol.,* **3,** No. 5.
1921, Further Observations on the Production of Parthenogenetic Frogs, *J. Gen. Physiol.,* **3,** No. 4.

1921, The Colloidal Behavior of Proteins, *J. Gen. Physiol.*, **3,** No. 4.

1922, Proteins and the Theory of Colloidal Behavior, McGraw-Hill Book Company, Inc., New York.

1922, Sodium Chloride and Selective Diffusion into Living Organisms, *J. Gen. Physiol.*, **5,** No. 2.

1922, The Explanation of the Colloidal Behavior of Proteins, *Science,* N. S., **56,** 369 and 731.

———, and F. W. Bancroft, 1912, Can the Spermatozoon Develop outside the Egg? *J. Exptl. Zoöl.*, **12,** No. 3.

———, and W. F. Ewald, 1913, Die Frequenz der Hertzätigkeitals eindeutige Funktion der Temperatur, *Biochem. Z.*, **58,** H. 3.

———, and M. Kunitz, 1923, Valency Rule and Alleged Hofmeister Series in the Colloidal Behavior of Proteins, *J. Gen. Physiol.*, **5,** No. 665 and 693.

———, and W. H. Lewis, 1902, On the Prolongation of the Life of the Unfertilized Eggs of the Sea Urchin by Potassium Cyanide, *Am. J. Physiol.*, **6,** No. 5.

———, and J. H. Northrup, 1917, On the Influence of Food and Temperature upon the Duration of Life, *J. Biol. Chem.*, **32,** No. 1.

1917, What Determines Duration of Life in Metazoa? *Proc. Nat. Acad. Sci.*, **3,** 382.

———, and H. Wasteneys, 1911, Sind die Oxydationsvorgänge die unabhängige Variable in den Lebenserscheinungen? *Biochem. Z.*, **36,** Nos. 5 and 6.

1912, Die Oxydationsvorgänge im befruchteten and unbefruchteten Seesternei, *Arch. Entwickl. Mech. Organ.*, **35,** H. 3.

Loeb, Leo, 1905, Blood Coagulation, *Med. News,* Apr. 1, 1905.

1909, Blood—Limulus, *Biochem. Z.*, **16,** 157.

Long, J. H., and Mary Hull, 1916, The Alleged Destruction of Trypsin by Pepsin and Acid, *J. Am. Chem. Soc.*, **38.**

1917, On the Optimum Reaction in Tryptic Digestion, *J. Am. Chem. Soc.*, **39,** 1051.

1917, The Normal Reaction of the Intestinal Tract, *J. Am. Chem. Soc.*, **39,** 1278.

Lucas, Keith, 1908, On the Rate of Development of the Excitatory Process in Muscle and Nerve, *J. Physiol.*, **37,** 459.

1908, The Temperature Coefficient of the Rate of Conduction in Nerve, *J. Physiol.*, **37,** 112.

1909, The All-or-none Contraction of Amphibian Skeletal Muscle, *J. Physiol.*, **38,** 113.

1910, An Analysis of the Changes and Differences in the Excitatory Process of Muscles and Nerves Based on the Physical Theory of Excitation, *J. Physiol.*, **40,** 225.

1912, *Croonian Lecture*—The Process of Excitation in Nerve and Muscle, *Proc. Roy. Soc. London,* **85B,** 495.

1916, On Summation of Propagated Disturbances in the Claw of Astacus, and on the Double Neuro-muscular System of the Adductor, *J. Physiol.*, **51,** 1.

1917, The Conduction of the Nervous Impulse, revised by E. D. Adrian, Longmans, Green & Co., London.

Lund, E. J., 1918 Intracellular Respiration, Relation of the State of Nutrition of Paramoecium to the Rate of Intracellular Oxidation, *Am. Jour. Physiol.*, **47,** No. 2.

1918, A Simple Method for Measuring Carbon Dioxide Produced by Small Organisms, *Biol. Bull.*, 36, No. 2.

Luscher, E., 1919, Gas Exchange and the Mechanical Work of the Frog's Heart, *Z. Biol.*, **70,** 245, abstract in *Chem. Abstracts,* **14,** 1139.

Lusk, Graham, 1917, The Elements of the Science of Nutrition, W. B. Saunders Company, Philadelphia.

1921, The Physiological Effect of Under-nutrition, *Physiol. Rev.*, **1,** No. 4. 523.

Lynch, V., 1919, The Function of the Nucleus of the Living Cell, *Am. J. Physiol.*, **48,** No. 2.

Lyon, E. P., 1918, Blood Pressure in Sharks and the Shock Problem, *Am. J. Physiol.*, **45,** 543.

Macallum, A. B., 1907, Ancestral Features in Blood Plasma of Vertebrates, *J. Biol. Chem.*, **7,** 11.

MacCallum, J. B., 1904, The Influence of Calcium and Barium on the Secretory Activity of the Kidney, *J. Exptl. Zoöl.*, **1,** No. 1.

MacCurdy, H., 1910, Degeneration in the Ganglion Cells of the Crawfish, *Cambarus bartonii, J. Comp. Neurol. Psych.*, **20,** No. 3.

Macdonald, J. S., 1909, Structure and Function of Striated Muscle, *Quart. J. Exptl. Physiol.*, **2,** 5.

MacDougal, D. T., 1918, Inhibition and Growth: The Growth Mechanism of Protoplasm, *Carnegie Inst. Yearbook, Wash.*, **17,** 57.

Madsen, T., and O. Wulff, 1918, Influence of Temperature on Phagocytosis, *Chem. Abstracts,* **12,** 815.

Marshall, E. K., and M. M. Crane, 1924, The Secretory Function of the Renal Tubules, *Am. J. Physiol.*, **70,** No. 3.

Martin, E. G., 1906, Absorption and Consumption of Oxygen by Heart Tissue, *Am. J. Physiol.*, **15,** 303.

1906, Relation of Inorganic Salts of Blood to Heart, *Am. J. Physiol.*, **16,** 191.

1913, Relation of Salts to Cardiac Contraction, *Am. J. Physiol.*, **32,** 165.

Mast, S. O., 1923, Mechanics of Locomotion in *Amoeba, Proc. Nat. Acad. Sci.*, **9,** 258.

———, and F. Root, 1916, Observations on *Amoeba* Feeding on Infusoria, *Proc. Nat. Acad. Sci.*, **2,** 188.

Mathews, A. P., 1921, Adsorption, *Physiol. Rev.*, **1,** 553.

Matill, H. I. and H. A., 1915, Digestive Process in *Limulus, J. Biol. Chem.*, **20,** 22.

Matula, J., 1912, Die Regulation der Atemrhythmik bei *Squilla mantes, Pflüger's Arch. ges. Physiol.*, **144,** 109.

1917, Untersuchungen über die Leistungen der Nervenzentern bei Dekapoden, *Pflüger's Arch. ges. Physiol.*, **169,** 503.

1918, Nerve Conduction in Cassipoea, *Carnegie Inst. Pub., Wash.*, No. 251.

1918, Nerve Conduction in Diluted and in Concentrated Sea Water, *Carnegie Inst. Pub., Wash.*, No. 252, 179.

1922, Hydrogen Ion Concentration and Electrical Conductivity of the Surface Water of the Atlantic and Pacific, *Carnegie Inst. Pub., Wash.*, No. 312, 61.

Mayet, L., 1912, Goitre et Cretinisme Endemiques, *Biol. J. Sci. Med.*, **2,** 13.

Mayor, A. G., 1906, Rhythmic Pulsation in Animals, *Carnegie Inst. Pub., Wash.*, Nos. 47 and 102 (1908).

1911, Converse Relation between Ciliary and Neuro-muscular Movement, *Carnegie Inst. Pub., Wash.*, No. 132.

1916, Theory of Nerve Conduction, *Proc. Nat. Acad. Sci.*, **2,** 37.

1917, Formula for Rate of Nerve Conduction in Sea Water, *Am. J. Physiol.*, **44.**

1917, Further Studies of Nerve Conduction in Cassiopea, *Am. J. Physiol.*, **42,** 469.

1917, Is Death from High Temperatures Due to Accumulation of Acid in the Tissues? *Proc. Nat. Acad. Sci.*, **3,** 602.

1917, Non-existence of Shell Shock in Fishes and Marine Invertebrates, *Proc. Nat. Acad. Sci.*, **3,** 597.

Maxwell, S. S., 1905, Effect of Salt Solutions on Ciliary Activity, *Am. J. Physiol.*, **13** 154.

1915, Relation of Salivary to Gastric Digestion, *Biochem. J.* **9,** 323.

McCarrison, R., 1918, The Thyroid Gland, Balliere, Tindall, and Cox, London.

McClendon, J. F., 1912, The Osmotic and Surface Tension Phenomena of the Living Elements and Their Physiological Significance, *Biol. Bull.*, **22,** No. 3.

1917, Diurnal Changes in the Sea at Tortugas, Florida, *Proc. Nat. Acad. Sci.*, **3.**

1917, The Direct and Indirect Calorimetry of Cassiopea, *J. Biol. Chem.*, **32,** 275.

1917, The Effect of Oxygen Tension on the Metabolism of Cassiopea, *Proc. Nat. Acad. Sci.*, **3.**

1917, The Effect of Stretching on the Rate of Conduction in the Neuro-muscular Network in Cassiopea, *Proc. Nat. Acad. Sci.*, **3,** 703.

1917, The Effect of Stretching on the Rate of Nerve Impulse, *J. Biol. Chem.*, **32,** No. 2.

1922, Are Iodides Foods? *Science*, N.S., **55,** No. 1423.

———, F. S. Bissell, and M. Lowe, 1920, Hydrogen Ion Concentration of the Contents of the Small Intestine, *J. Am. Med. Assoc.*, **75,** 1638.

——, C. C. Gault, and S. Mulholland, 1917, The Hydrogen Ion Concentration, Carbon Dioxide Tension, and Carbon Dioxide Content of Sea Water, *Carnegie Inst. Pub., Wash.*, No. 351, 21.

———, and P. H. Mitchell, 1912, How Do Isotonic Sodium Chloride Solution and Other Parthenogenic Agents Increase Oxidation in the Sea Urchin's Egg? *J. Biol. Chem.*, **10,** No. 6.

———, A. Shedlov, and B. Karpman, 1918, Hydrogen Ion Concentration in Small Intestine, *J. Biol. Chem.*, **34,** 1.

McCord, C. P., 1915, The Pineal Gland, *Interstate Med. J.*, **22,** No. 4.

McCrudden, F. H., 1922, The Determination of the Hydrogen Ion Concentration, *Pub. Health Repts.*, No. 730, 334.

McDowell, R. J. S., 1917, A Cross-striated Mammalian Muscle Preparation, *Quart. J. Exptl. Physiol.*, **11,** 381.

McIndoo, N. E., 1917, The Olfactory Organs of Lepidoptera, *J. Morphology*, **29,** 33.

McIntosh, W. A., 1918, Histological Study of Fat Contained in the Mucosa of the Alimentary Tract of Moderately Starved Cats, *Am. J. Physiol.*, **46,** 570.

Meek, W. J., and J. A. E. Eyster, 1916, Origin of Cardiac Impulse in Turtle's Heart, *Am. J. Physiol.*, **39,** 291.

———, and H. S. Gasser, 1918, Blood Volume: Method for Determination, etc., *Am. J. Physiol.*, **47,** 302.

———, and W. E. Leaper, 1911, Effect of Pressure on Conductivity in Nerve and Muscle, *Am. J. Physiol.*, **27,** 308.

Meigs, E. B., 1905, A Mechanical Theory of Muscular Contraction and Facts Supporting It, *Am. J. Physiol.*, **14,** 138.

1910, Effects of Water and Various Solutions on Weight and Length of Striated Muscle, *Am. J. Physiol.*, **26,** 191.

1912, Microscopic Studies on Living Smooth Muscle, *Am. J. Physiol.*, **29,** 317.

1912, The Structure of Smooth Muscle and Its Response to Distilled Water, and Hypertonic Salt Solutions, *Quart. J. Exptl. Physiol.*, **5,** No. 1.

1915, The Osmotic Properties of Different Kinds of Muscle, *Science*, N. S., **41,** 689.

Mendel, L. B., and H. G. Wells, 1909, Experimental Studies on the Physiology of Molluscs, *Am. J. Physiol.*, **24,** 170.

Mesnil, F., 1901, Recherches sur le Digestion intracellulaire et les Diastases des Actinies, *Ann. inst. Pasteur*, **15,** 352.

Metcalf, M. M., 1907, Excretory Organs of *Opalina*, *Arch. Protistenk.*, **10,** 183 and 365.

Meyer, A. L., 1917, A New Method of Obtaining the Respiratory Gases in Animals and Some of the Results, *Am. J. Physiol.*, **45,** 16.

Meyerhoff, O., 1919, The Respiration of the Frog Muscle, *Pflüger's Arch. ges. Physiol.*, **175,** 20, abstract in *Chem. Abstracts*, **13,** 3227.

Miller, F. R., 1910, On the Rhythmical Contractility of the Anal Musculature of Crayfish and Lobster, *J. Physiol.*, **40,** 431.

Millikan, R. A., 1921, The Electron, University of Chicago Press.
Mills, T. Wesley, 1886, Notes on the Urine of the Tortoise with Special Reference to Uric Acid and Urea, *J. Physiol.*, **7,** 453.
Mines, G. R., 1913, On Dynamic Equilibrium in the Heart, *J. Physiol.*, **46,** 349.
1913, On Functional Analysis by the Action of Electrolytes, *J. Physiol.*, **46,** 188.
1913, On the Summation of Contractions, *J. Physiol.*, **46,** 1.
Mitchell, H. H., 1924, The Nutritive Value of Proteins, *Physiol. Rev.*, **4,** No. 3.
Mitchell, P. H., 1912, The Oxygen Requirements of Shellfish, *Bull. U. S. Bur. Fish.*, **32.**
1923, Textbook of General Physiology, McGraw-Hill Book Company, Inc., New York.
Moore, A. R., 1915, On the Rhythmical Susceptibility of Developing Sea-urchin Eggs to Hypertonic Sea Water, *Biol. Bull.*, **28, No. 5.**
1916, Cryoscopic Measurements of the Osmotic Differences between Resting and Fatigued Muscle, *Am. J. Physiol.*, **41.**
1917, Chemical Differentiation of the Central Nervous System in Invertebrates, *Proc. Nat. Acad. Sci.*, **3,** 598.
1917, The Mechanism of Cytolysis in Sea Urchin Eggs, *J. Biol. Chem.*, **28,** No. 2.
1918, Reversal of Reaction by Means of Strychnine in Planarians and Starfish, *J. Gen. Physiol.*, **1,** 1.
1918, The Carbon Dioxide of Injury and of Respiration, *Proc. Soc. Exptl. Biol. Med.*, **16,** 35.
1919, The Respiratory Rate of the Sciatic Nerve of the Frog in Rest and Activity, *J. Gen. Physiol.*, **1,** 613.
1919, The Selective Action of Nicotine on the Central Nervous System of the Squid, *Loligo pealii*, *J. Gen. Physiol.*, **1,** 505.
1921, Chemical Stimulation of the Annelid Nerve Cord, *Proc. Soc. Exptl. Biol. Med.*, **18,** 213.
1923, The Function of the Brain in Locomotion of the Polyclad Worm, *Yungia aur.*, *J. Gen. Physiol.*, **6,** 73.
1924, The Nervous Mechanism of Coordination in the Crinoid *Antedon rosaceus*, *J. Gen. Physiol.*, **6,** 281.
———, and F. M. Kellogg, 1916, Note on the Galvanotropic Response of the Earthworm, *Biol. Bull.*, **30.**
Moore, Benjamin, 1921, Biochemistry, Edward Arnold and Company, London.
———, Edie, Whitley, and Dakin, 1912, Nutrition and Metabolism of Marine Animals in Relation to (*a*) Dissolved Organic Matter, and (*b*) Particulate Organic Matter of Sea Water, *Biochem. J.*, **6,** 255.
———, E. Whitley, and E. S. Edie, 1912, Nutrition, Metabolism, and Respiration of Aquatic Animals, *Brit. Assoc. Advancement Sci., Report Eighty-second Meeting*, 654.
———, Whitley, and Webster, 1912, The Basic and Acidic Proteins of the Sperm of *Echinus esculentus*—Direct Determination of the Osmotic Pressure of a Protamine or Histone, *Biochem. J.*, **7,** 142.
Moore, C. R., 1916, On the Superposition of Fertilization on Parthenogenesis, *Biol. Bull.*, **31,** No. 3.
1917, On the Capacity for Fertilization after the Initiation of Development, *Biol. Bull.*, **33,** No. 4.
Moore, E. L., 1919, The Effect of Adrenin upon the Rate of Locomotion of *Planaria* and of Toad Larvae, *Biol. Bull.*, **37,** No. 3.
Moore, Mary M., 1918, Evidence for the Enzymatic Basis of Heart Beat, *Am. J. Physiol.*, **45,** 188.

Morgulis, S., 1915, The Body Surface of Flounders and Its Relation to the Gaseous Metabolism, *Am. J. Physiol.*, **36,** 207.

1919, Studies on the Nutrition of Fish, Experiments upon Brook Trout, *J. Biol. Chem.*, **37,** 391.

Morse, H. N., 1914, The Osmotic Pressure of Aqueous Solutions, *Carnegie Inst. Pub., Wash.*, No. 198.

Myers, F. J., and J. F. McClendon, 1920, Hydrogen Ion Concentration of Human Duodenum, *J. Biol. Chem.*, **41.**

Nelson, J. A., 1917, The Relation of the Malpighian Tubules of the Hind Intestine in the Honeybee Larva, *Science*, N. S., **46,** 343.

Neuhausen, B. S., 1922, The Solubility of Gases in Liquids, *J. Phys. Chem.*, **26,** 553.

Newman, H. H., 1905, On Some Factors Governing the Permeability of the Egg Membrane by the Sperm, *Biol. Bull.*, **9,** No. 6.

1906, Respiration of the Heart (Limulus), *Am. J. Physiol.*, **15,** 371.

Nolf, P., 1906–1908, Contribution a l'etude de la coagulation du sang, *Arch. intern. physiol.*, **4,** 165; **6,** 1, 115, 306; **7,** 280, 379, 411.

Northrop, J. H., 1926, The Resistance of Living Organisms to Digestion by Pepsin or Trypsin, *J. Gen. Physiol.*, **9,** 497.

Okada, S., 1916, On the Optimal Reaction of Pepsin, *Biochem. J.*, **10,** 126.

Olmstead, J. M. D., 1922, The Rôle of the Nervous System in the Locomotion of Certain Marine Polyclads, *J. Exptl. Zoöl.*, **36,** No. 1.

Orton, J. H., 1913, Ciliary Mechanisms of Gill in Amphioxus, Tunicates, and Lamellibranchs, *J. Mar. Biol. Assoc.*, **10,** 19.

1913, Ciliary Mechanisms in Brachiopods, *loc. cit.*, **10,** 283.

Osborne, H. F., 1917, Application of Laws of Action, Reaction, and Interaction in Life Evolution, *Proc. Nat. Acad. Sci.*, **3,** 8.

Osborne, W. A., 1906, Action of Carbon Dioxide on Respiration of Goldfish, *Biochem. J.*, **1,** 377.

Osterhout, W. J. V., 1907, On Nutrient and Balanced Solutions, *Univ. Calif. Pub., Bot.*, **2,** No. 15.

1909, On the Penetration of Inorganic Salts into Living Protoplasm, *Z. physik. Chem.*, **70.**

1909, The Nature of Balanced Solutions, *Bot. Gaz.*, **47,** 48.

1912, The Permeability of Protoplasm to Ions and the Theory of Antagonism, *Science*, N. S., **35,** 112.

1916, The Penetration of Balanced Solutions and the Theory of Antagonism, *Science*, N. S., **44,** No. 1133.

1917, Antagonism and Permeability, *Science*, N.S., **45,** 97.

1918, The Nucleus as a Center of Oxidation, *Brooklyn Bot. Gard. Mem.*, **1,** 342.

Ostwald, Wolfgang, 1922, Theoretical and Applied Colloid Chemistry, translated by M. H. Fischer, John Wiley & Sons, New York.

Packard, Charles, 1914, The Effect of Radium Radiations on the Fertilization of Nereis, *J. Exptl. Zoöl.*, **16,** No. 1.

Parhon, Mlle., 1910, Respiration Experiments on Bees during Four Seasons, *Ann. Sci. Nat. Zoöl.*, **9,** abstract in *Chem. Abstracts*, **4,** 1330.

Parker, G. H., 1905, Ciliary Reversal in Metazoans, *Am. J. Physiol.*, **13,** 13.

1905, Reversal of Effective Stroke of Labial Cilia of Sea Anemones by Organic Substances, *Am. J. Physiol.*, **14,** 1.

1909, A Mechanism for Organic Correlation, *Am. Naturalist*, **43,** 212.

1909, The Origin of the Nervous System and Its Appropriation of Effectors, *Pop. Sci. Monthly*, **75,** 56, 137, 253, and 338.

1910, The Reactions of Sponges, with a Consideration of the Origin of the Nervous System, *J. Exptl. Zoöl.*, **8,** 1.

1911, The Origin and Significance of the Primitive Nervous System, *Proc. Am. Phil. Soc*, **1,** 217.

1916, The Effector Mechanisms of Actinians, *J. Exptl. Zoöl.*, **21,** No. 4.

1916, The Structure of *Metridium marginatus* with Special Reference to Its Neuromuscular Mechanism, *J. Exptl. Zoöl.*, **21,** No. 4.

1917, Nervous Transmission in the Actinians, *J. Exptl. Zoöl.*, **22,** No. 1.

1918, The Rate of Transmission in the Nerve Net of the Coelenterates, *J. Gen. Physiol.*, **1,** No. 2.

1919, The Elementary Nervous System, J. B. Lippincott Company, Philadelphia.

1922, The Excretion of Carbon Dioxide by Relaxed and Contracted Sea Anemones, *J. Gen. Physiol.*, **5,** No. 1.

— —, and E. G. Titus, 1916, Neuro-muscular Apparatus of Sea Anemone, *Proc. Nat. Acad. Sci.*, **2,** 339.

Parsons, T. R., 1917, The Reaction of the Blood in the Body, *J. Physiol.*, **51,** 440.

Patterson, I. T. L., 1916, Contributions to the Physiology of the Stomach, *Am. J. Physiol.*, **42,** 56.

Patterson, S. W., H. Piper, and E.H. Starling, 1914, The Regulation of the Heart Beat, *J. Physiol.*, **48,** 465.

Paul, J. H., and J. S. Sharpe, 1916, Calcium Metabolism, *J. Physiol.*, **50,** 183.

Pembrey, M. S., 1894, On the Reaction Time of Mammals to Changes in the Temperature of Their Surroundings, *J. Physiol.*, **15,** 401.

1903, Further Observations upon the Respiratory Exchange and Temperature of Hibernating Mammals, *J. Physiol.*, **29,** 195.

———, and W. H. White, 1896, The Regulation of Temperature in Hibernating Animals, *J. Physiol.*, **19,** 477.

Peters, R. A., 1913, Heat Production of Fatigue and Its Relation to Lactic Acid Production in Amphibian Muscle, *J. Physiol.*, **47,** 243.

Philip, J. C., 1914, Physical Chemistry, Its Bearing on Biology and Medicine, 312 pp., 2d ed., Edward Arnold and Company, London.

Pieron, H., 1913, On the Way in Which Octopus Feeds upon Its Prey, Particularly Lamellibranchs, *Arch. Zoöl. Exp.*, **53,** Notes and Rev. 1.

Pike, F. H., H. C. Coombs, and A. B. Hastings, 1919, The Dependence of Respiratory Activity upon Conditions in the Central Mechanism, *Am. J. Physiol.*, **49,** 125.

— —, and E. L. Scott, 1915, The Significance of Certain Internal Conditions of the Organism in Organic Evolution, *Am. Naturalist*, **49,** 321.

Plimmer, R. H. A., 1908, The Chemical Constitution of the Proteins, Longmans, Green & Co., London.

Polimanti, O., 1913, Beiträge zur Physiologie vom *Maja verrucosa*, *Arch. Anat. Physiol., Physiol. Abstracts*, **S117.**

Porter, E. L., and V. W. Hart, 1923, Reflex Contractions of an All-or-none Character in the Spinal Cat, *Am. J. Physiol.*, **66,** No. 2.

Portier, P., 1908, Temperature de Marine Invertebrates, *C. R. Soc. Biol. Paris*, **64,** 400.

1910, The Osmotic Pressures of the Liquids of the Marine Mammals and Birds, *Inst. Ocean. Monaco, Bull.*, 180.

Pratt, E. M., 1905, Digestive Organs of Alcyonaria, *Quart. J. Med. Sci.*, **49,** 327.

Pratt, F. H., 1917, The All-or-none Principle in Graded Response of Skeletal Muscle, *Am. J. Physiol.*, **44,** 517.

——, and J. P. Eisenberger, 1919, Quantal Phenomena in Sartorius of Rana, *Am. J. Physiol.*, **49,** 1.

Przibram, Hans, 1908, Experimental Zoölogy, Part I. Embryogeny, Cambridge University Press.

Purser, G. L., 1914, Preliminary Notes on Some Problems Connected with Respiration in Insects Generally, and Aquatic Forms in Particular, *Proc. Cambridge Phil. Soc.*, **18,** 63.

Pütter, A., 1911, Vergleichende Physiologie, Gustav Fischer, Jena.

Reed, G. B., 1915, The Rôle of the Oxidases in Respiration, *J. Biol. Chem.*, **22,** No. 1.

Regnault-Riset, 1849, La Respiration des Animaux, Paris.

Reichert, E. T., and A. P. Brown, 1908, Crystallographic Study of Haemoglobin, *Proc. Am. Phil. Soc.*, **47,** 298.

Reuss, H., 1910, Action of Carbon Dioxide in Respiration of Lower Vertebrates, Especially Fish, *Chem. Abstracts,* **4,** 1627.

Rhumbler, L., 1912, Mechanics of the Cell and Mechanism of Development, *Brit. Assoc. Advancement Sci., Rept. Eighty-second Meeting,* 499.

Richards, A. N., and O. H. Plant, 1917, Relation of Blood Pressure to Urine Formation, *Am. J. Physiol.*, **42,** 592.

Riddle, O., 1909, The Rate of Digestion in Cold-blooded Vertebrates, *Am. J. Physiol.*, **4,** 24 and 447.

1924, A Hitherto Unknown Function of the Thymus, *Am. J. Physiol.*, **68,** No. 3.

——, and P. Frey, 1925, The Growth and Age Involution of the Thymus in Male and Female Pigeons, *Am. J. Physiol.*, **71,** No. 2.

Ringer, S., 1880 1882, Concerning the Influence Exerted by Each of the Constituents of the Blood on the Contraction of the Ventricle, *J. Physiol.*, **3,** 380.

1882–1883, A Further Contribution Regarding the Influence of the Different Constituents of the Blood on the Contractions of the Heart, *J. Physiol.*, **4,** 29.

1882–1883, A Third Contribution Regarding the Influence of the Inorganic Constituents of the Blood on the Ventricular Contraction, *J. Physiol.*, **4,** 222.

1886, Further Experiments Regarding the Influence of Small Quantities of Lime, Potassium, and Other Salts on Muscular Tissue, *J. Physiol.*, **7,** 291.

Roaf, H. E., 1906, Digestive Glands in Mollusca and Decapod Crustacea, *Biochem. J.*, **1,** 390.

1906, Note on the Osmotic Effect of Various Strengths of Different Salts upon Cell Volume, *Biochem. J.*, **1,** 383.

1908, Calorimetric Method to Show Activity of Either Peptic or Tryptic Enzymes, *Biochem. J.*, **3,** 188.

1908, Hydrolytic Enzymes of the Invertebrates, *Biochem. J.*, **3,** 462.

1910, Contributions to the Physiology of the Marine Invertebrates, *J. Physiol.*, **39,** 438.

1914, The Vapor Pressure Hypothesis of Contractions of Striated Muscle, *Proc. Roy. Soc. London,* **88B,** 139.

1914, Time Relations of Acid Production in Muscle During Contraction, *J. Physiol.*, **48,** 380.

——, and M. Nierenstein, 1907 Physiological Action of Extracts, of Hypobranchial Gland of *Purpura lapillus*, *J. Physiol.*, **36,** 5.

Robertson, T. B., 1912, Amoeboid Motion and Protoplasmic Streaming in Same Model, *Science,* N. S., **36,** 446.

1920, Principles of Biochemistry, Lea & Febiger, Philadelphia.

1920, The Physical Chemistry of the Proteins, Longmans, Green & Co., New York.

Rogers, C. G., 1905, The Effect of Various Salts upon the Survival of the Invertebrate Heart, *J. Exptl. Zoöl.*, **2,** No. 2.

1911, Studies upon the Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals, *Am. J. Physiol.*, **28,** No. 11.

——, and K. S. Cole, 1925, Heat Production by the Eggs of *Arbacia punctulata* During Fertilization and Early Cleavage, *Biol. Bull.*, **49,** 338.

——, and E. M. Lewis, 1914, The Relation of the Body Temperature of the Earthworm to That of Its Environment, *Biol. Bull.*, **27,** No. 5.

1914, The Temperature Coefficient of the Rate of Contraction of the Dorsal Blood Vessel of the Earthworm, *Biol. Bull.*, **27,** No. 5.

Saguchi, S., 1917, Studies on Ciliated Cell, *J. Morphology*, **29,** No. 1.

Sanderson, E. D., 1910, Relation of Temperature and Growth of Insects, *J. Econ. Entomol.*, **3,** 113.

Sanford, E. W., 1915, Experiments on Physiology of Digestion in Blattidae, *Proc. Soc. Exptl. Biol. Med.*, **13,** 8, 193.

1918, Experiments on the Physiology of Digestion in the Blattidae, *J. Exptl. Zöol.*, **25,** 355.

Schaeffer, A. A., 1910, Selection of Food in Stentor, *J. Exptl. Zoöl.*, **8,** 75.

1920, Amoeboid Movement, 156 pp., Princeton University Press.

Schäfer, E. A., 1898, Textbook of Physiology, The Macmillan Company, London.

1912, Life: Its Nature, Origin, and Maintenance, address before British Association for the Advancement of Science, Longmans, Green & Co., London.

1916, The Endocrine Organs, Longmans, Green & Co., London.

Schlüter, C., 1911–1912, Beiträge zur Physiologie und Morphologie des Verdauungsapparatus der Insekten, *Z. allgem. Physiol.*, **13,** 155.

Schwanecke, G., 1913, Blutgefasssystem von *Anodonta*, *Z. wiss. Zoöl.*, **107,** 1.

Schwitalla, A. M., 1924, The Influence of Temperature on the Rate of Locomotion in *Amoeba*, *J. Morph. Physiol.*, **39,** No. 2.

Scott, E. L., 1913, The Reaction of Pancreatic Extract to the Sugar of the Blood, *Proc. Soc. Exptl. Biol. Med.*, **10.**

Scott, F. H., 1915, Mechanism of Fluid Absorption from Tissue Spaces, *J. Physiol.*, **50,** 57.

Scott, G. G., 1913, Some Effects on *Fundulus* of Changes in the Density of the Surrounding Water, *Biol. Bull.*, **25,** 121.

1916, The Evolutionary Significance of the Osmotic Pressure of the Blood, *Am. Naturalist*, **50.**

———, and W. E. Kellicott, 1917, Oxygen Consumption During Development of *Fundulus heteroclitus*, *Anat. Record*, **11,** 531.

——, and G. F. White, 1910, Permeability to Salts of Gill Membranes of Fish, *Science*, N. S., **32,** 767.

Scott, R. W., 1918, Significance of Undissociated Carbon Dioxide, *Am. J. Physiol.*, **47,** 43.

Seelye, A. B., 1906, Circulation and Respiratory System of *Desmognathus*, *Proc. Soc. Nat. Hist.*, **32,** 335.

Sellier, J., 1906, Digestion in Helix, *C. R. Soc. Biol. Paris*, **61,** 205.

1907, Milk Curdling and Proteolytic Action of Digestive Juice of Cephalopods, *C. R. Soc. Biol. Paris*, **63,** 703 and 705.

1909, Identity of Proteolytic Ferment and Rennin, *C. R. Soc. Biol. Paris*, **65,** 754.

Sharp, L. W., 1921, Introduction to Cytology, McGraw-Hill Book Company, Inc., New York.

Sharpe, N. C., 1912, Urine Secretions in Birds, *Am. J. Physiol.*, **31,** 75.

Shelford, V. E., and W. C. Allee, 1913, Reactions of Fishes to Atmospheric Gases, *J. Exptl. Zoöl.*, **14,** 207.

Sherman, H. C., 1921, The Vitamins, *Physiol. Rev.*, **1,** 4, 598.

Simpson, S., 1911, Heat Regulation in the Woodchuck, *Am. J. Physiol.*, **29,** 12.

1911, Observations on Body Temperatures of Some Swimming and Diving Birds, *Proc. Roy. Soc. Edinburgh*, **32,** 19.

Smallwood, W. M., and C. G. Rogers, 1907, Some Observations on the Cytology of Invertebrate Nerve Cells, *Proc. Seventh Intern. Zoöl. Congress. Cambridge, U. S. A.*, The University Press.

1908, The Molluscan Nerve Cell, etc., *J. Comp. Neurol. Psych.*, **18,** No. 1.

1909, The Comparative Cytology and Physiology of Some of the Metabolic Bodies in the Cytoplasm of Invertebrate Nerve Cells, *Folia Neuro-biol.*, **3.**

1910, Some Metabolic Bodies in the Cytoplasm of Nerve Cells of Gastropods, a Cephalopod, and an Annelid, *Anat. Anz.*, **36,** Nos. 8 to 10.

Smith, G., 1909, Haemoglobin, *Nature*, **81,** 395.

1915, Physiology of Growth and Reproduction in Crustacea, *Proc. Roy. Soc. London*, **88,** 418.

Smith, H. W., and G. H. A. Clowes, 1924, Influence of Hydrogen Ion Concentration on the Development of Normally Fertilized *Arbacia* and *Asterias* Eggs, *Biol. Bull.*, **47,** No. 6.

Smith, P. E., 1918, Growth of Normal and Hypophysectomized Tadpoles as Influenced by Endocrine Diets, *Univ. Calif. Pub. Phys.*, **5,** 11.

Snodgrass, R. E., 1925, The Anatomy and Physiology of the Honeybee, McGraw-Hill Book Company, Inc., New York.

Snyder, C. D., 1905, On the Influence of Temperature upon Cardiac Contraction and Its Relation to Influence of Temperature upon Chemical Reaction Velocity, *Univ. Calif. Pub. Phys.*, **2,** 125.

1906, The Influence of Temperature upon the Rate of the Heart Beat in the Light of the Law for Chemical Reaction Velocity, *Am. J. Physiol.*, **17,** No. 4.

1908, Comparative Study of Temperature Coefficients of Velocities of Various Physiological Actions, *Am. J. Physiol.*, **22,** 309.

1908, Der Temperaturkoefficient der Resorption bei tierischen Membranen, *Zentr. Physiol.*, **22,** 236.

1908, The Temperature Coefficient of the Velocity of Nerve Conduction, *Am. J. Physiol.*, **22,** No. 1.

1911, On the Meaning of Variations in the Magnitude of the Temperature Coefficients of Physiological Processes, *Am. J. Physiol.*, **28,** 167.

1913, Is the Rate of the Surviving Mammalian Heart a Linear or an Exponential Function of the Temperature? *Z. allgem. Physiol.*, **15,** H.1 and 2.

1914, Is the Contraction of Smooth Muscle Accompanied by Heat Production? *Am. J. Physiol.*, **35,** No. 3.

1915, A Study of the Causes of Respiratory Change of Heart Rate, *Am. J. Physiol.*, **37,** No. 1.

1916, Is the Contraction of Smooth Muscle Accompanied by Heat Production? *Am. J. Physiol.*, **40,** 138.

1917, The Heat Liberated by the Beating Heart, *Am. J. Physiol.*, **44,** No. 3.

1918, The Heat Liberated by the Beating Heart, *Am. J. Physiol.*, **47,** 156.

1920, Heat Production in Turtle's Cardia in Response to Stimulus through Vagus Nerve, *Am. J. Physiol.*, **51,** No. 1.

1922, Heat Liberated by the Beating Heart, *Am. J. Physiol.*, **59,** No. 1.

1923, On Sources of Heat in Muscle Contraction, *Am. J. Physiol.*, **66,** No. 2.

1923, The Heat Liberated from the Beating Heart, *Am. J. Physiol.*, **63,** No. 3.

———, and E. C. Andrus, 1919, On the Relation between Tonus and Smooth Muscle in Terrapin Heart, *J. Pharmacol. Exptl. Therap.*, **14,** No. 1.

Southworth, F. C., and A. C. Redfield, 1926, The Transport of Gas by the Blood of the Turtle, *J. Gen. Physiol.*, **9,** 387.

Spaeth, R. A., 1913, The Mechanism of the Contraction in Melanophores of Fishes, *Anat. Anz.*, **44,** Nos. 20 and 21.

1916, Evidence Proving the Melanophore to be a Disguised Type of Smooth Muscle Cell, *J. Exptl. Zoöl.*, **20,** No. 2.

1916, The Responses of Single Melanophores to Electrical Stimulation, *Am. J. Physiol.*, **41,** No. 5.

1917, The Physiology of the Chromotophores of Fishes, *Am. J. Physiol.*, **42,** 595.

Speidel, C. C., 1925, Studies of Hyperthyroidism, *Anat. Record*, **31,** No. 1.

Springer, M. G., 1922, The Effect upon Developing Eggs of Extracts of Embryos of the Same Species, *Biol. Bull.*, **43,** No. 2.

Starling, E. H., 1918, The Law of the Heart, *Linacre Lecture*, Longmans, Green & Co., London.

Stockard, C. R., 1909, Growth, *Carnegie Inst. Pub., Wash., Eighth Yearbook*, 133.

1921, Developmental Rate and Structural Expression: An Experimental Study of Twins, Double Monsters, and Single Deformities, and the Interaction among Embryonic Organs During Their Origin and Development, *Am. J. Anatomy*, **28,** No. 2.

Sullivan, M. X., 1905, Physiology of the Digestive Tract of Elasmobranchs, *Am. J. Physiol.*, **15,** 42.

Swingle, W. W., 1918, Iodine as the Active Principle of the Thyroid Gland, *Assoc. Study Internal Secretions, Bull.*, **2,** No. 3.

1919, Iodine and the Thyroid—The Specific Action of Iodine in Accelerating Amphibian Metamorphosis, *J. Gen. Physiol.*, **1,** No. 6.

1919, The Effects of Feeding Iodine to Normal and Thyroidectomized Tadpoles, *J. Exptl. Zoöl.*, **27,** No. 3.

1921, The Relation of the Pars Intermedia of the Hypophysis to Pigmentation Changes in Anuran Larvae, *J. Exptl. Zoöl.*, **34,** No. 2.

1922, Iodine and Anuran Metamorphosis, *Science*, N. S., **56,** 720.

1922, Is There a Transformation of Sex in Frogs? *Am. Naturalist*, **56.**

1922, Spontaneous Metamorphosis of the American Axolotl, *Am. Naturalist*, **56,** December, 1922.

1923, Iodine and Amphibian Metamorphosis, *Biol. Bull.*, **45,** No. 5.

1923, Thyroid Transplantation and Anuran Metamorphosis, *J. Exptl. Zool.*, **37,** No. 2.

1925, Autoplastic and Homoplastic Parathyroid Transplantation, *Am. J. Anatomy*, **36,** No. 1.

——, O. M. Helff, and R. L. Zwemer, 1924, The Effect of Thyroxin and Its Acetyl Derivative on Amphibians and Mammals, *Am. J. Physiol.*, **70,** No. 1.

Symes, W. L., 1914 and 1922, Apparatus for Perfusion of Frog's Heart, *J. Physiol.*, **48,** 25; **52,** 46.

Tait, J., 1910, Coagulation in the Amphipod, *Gammarus*, *J. Physiol.*, **40,** 41.

1910, Crustacean Blood Coagulation as Studied in the Arthrostraca, *Quart. J. Exptl. Physiol.*, **3,** 1.

1911, Types of Crustacean Blood Coagulation, *J. Mar. Biol. Assoc.*, **9,** 191.

1918, Capillary Phenomena Observed in Blood Cells: Thigmocytes, Phagocytosis, Amoeboid Movements, Differential Adhesiveness of Corpuscles, Emigration of Leucocytes, *Quart. J. Exptl. Physiol.*, **12,** 1.

——, and J. D. Gunn, 1918, The Blood of *Astacus fluviatilis*, *Quart. J. Exptl. Physiol.*, **12,** 35.

Takamine, Jokichi, 1901, The Isolation of the Active Principle of the Suprarenal Gland, *J. Physiol.*, **27,** 29.

TAKENUCHI, T., and R. INONE, 1910, On the Existence of an Enzyme in the Silkworm Which Produces Ammonia as a Cleavage Product of Amino-compounds, *Seventh Intern. Cong. App. Chem.*, **2,** 104.

TALBERT, G. A., 1920, Hydrogen Ion Concentration of Urine as the Result of Work and Heat, *Am. J. Physiol.*, **50,** 579.

TASHIRO, S., 1914, Metabolism in Ganglionated Cord of King Crab (Limulus) at Tortugas, *Carnegie Inst. Pub., Wash., Thirteenth Yearbook*, 216.

1915, The Nature of the Nervous Impulse, *Proc. Nat. Acad. Sci.*, **1,** 110.

———, and H. S. ADAMS, 1914, Comparison of Carbon Dioxide Output of Nerve Fibers and Ganglia in Limulus, *J. Biol. Chem.*, **18,** 329.

TAYLOR, A. E., 1911, On the Conception and Definition of Term Catalyzer, *J. Biol. Chem.*, **8,** 503.

TAYLOR, C. V., 1919, The Neuromotor System of *Euplotes*, *Proc. Soc. Exptl. Biol. Med.*, **16,** 101.

1923, The Contractile Vacuole in *Euplotes:* An Example of the Sol-gel Reversibility of Cytoplasm, *J. Exptl. Zoöl.*, **37,** No. 3.

TENNENT, D. H., 1913, Echinoderm Hybridization, *Science*, N. S., **37,** 535.

TILNEY, F., and L. F. WARREN, 1919, The Morphology and Evolutionary Significance of the Pineal Body, Wistar Institute of Anatomy and Biology.

1923, Investigations on the Hybridization of Echinoids, *Carnegie Inst. Pub., Wash., Twenty-second Yearbook*, 169.

1924, Specificity in Fertilization, *Science*, N. S., **60,** 162.

TINKER, F., 1916, The Microscopic Structure of Semi-permeable Membranes and the Part Played by Surface Forces in Osmosis, *Proc. Roy. Soc. London*, **92A,** 357.

TRUE, R. H., 1915, Antagonism and Balanced Solutions, *Science*, N. S., **41,** 653.

UNWIN, E. E., 1908, Respiration of Land Isopods, *Brit. Assoc. Advancement Sci., Rept. Seventy-eighth Meeting*, 736.

VAN DER HYDE, H. C., 1921, Studies on Organic Regulation, *J. Biol. Chem.*, **46,** No. 3.

1922, On the Physiology of Digestion, Respiration, and Excretion in Echinoderms, C. DeBoer, Jr., Den Helder.

1922, On the Respiration of *Dytiscus marginalis*, *J. Exptl. Zoöl.*, **35,** No. 3.

VAN SLYKE, D. D., 1921, The Carbon-dioxide Carriers of the Blood, *Physiol. Rev.*, **1,** 141.

———, and G. F. WHITE, 1910, Digestion of Protein in Stomach and Intestine of Dogfish, *Proc. Soc. Exptl. Biol. Med.*, **8,** 11.

VERNON, H. M., 1895, Respiratory Exchanges of the Lower Marine Invertebrates, *J. Physiol.*, **19,** 18.

1908, Intracellular Enzymes, John Murray, London.

1910, Respiration of Tortoise Heart in Relation to Functional Activity, *J. Physiol.*, **40,** 295.

1913, Autocatalysis of Trypsin, *J. Physiol.*, **47,** 325.

1914, The Activation of Trypsinogen, *Biochem. J.*, **8,** 494.

VERZAR, F., 1912, Influence of Lack of Oxygen on Tissue Respiration, *J. Physiol.*, **45,** 39.

VINCENT, SWALE, 1922, Internal Secretions and the Ductless Glands, Edward Arnold and Company, London.

WARBURG, O., 1908, Beobachtungen über die Oxidationsprozesses im Seeigelei, *Z. Phys. Chem.*, **57,** 1.

WASHBURN, E. W., 1921, Principles of Physical Chemistry, McGraw-Hill Book Company, Inc., New York.

Weinland, E., V. L. Gibbons, and A. Adam, 1918, Beobachtungen über den Gaswechsel von Anodonta cygnea, *Z. Biol.*, **69.**

Weizsacker, V., 1914, Myothermic Experiments in Salt Solutions in Relation to the Various Stages of a Muscular Contraction, *J. Physiol.*, **48,** 396.

Wells, M. M., Resistance of Fishes to Different Concentrations and Combinations of Oxygen and Carbon Dioxide, *Biol. Bull.*, **25,** 323.

Wiggers, C. J., 1911, The Physiology of the Pituitary Gland and the Actions of Its Extracts, *Am. J. Med. Sci.*, April, 1911.

Willem, V., 1917, Le Mechanisme de la filtration dans les Solenosomes des nephridies, *Arch. Néerland physiol.*, **2,** 182.

Williams, L. W., 1907, The Structure of Cilia, Especially in Gastropods, *Am. Naturalist*, **41,** 545.

1908, Physiology of the Stomach of Higher Crustacea, *Science*, N. S., **27,** 490.

Willier, B. H., 1924, The Endocrine Glands and the Development of the Chick, *Am. J. Anatomy*, **33,** No. 1.

———, L. H. Hyman, and S. A. Rifenburgh, 1925, A Histochemical Study of Intracellular Digestion in the Triclad Flatworms, *J. Morph. Physiol.*, **40,** No. 2.

Willis, H. S., 1916, The Influence of the Nucleus on the Behavior of *Amoeba*, *Biol. Bull.*, **30,** 253.

Willows, R. S., and E. Hatschek, 1919, Surface Tension and Surface Energy, 2d ed., P. Blakiston's Son and Company, Philadelphia.

Wodehouse, R. B., 1917, Direct Determinations of Permeability, *J. Biol. Chem.*, **29,** 453.

Woodruff, L. L., and W. W. Swingle, 1924, The Effects of Thyroid and Some Other Endocrine Products on *Paramoecium*, *Am. J. Physiol.*, **69,** No. 1.

Woodworth, R. S., 1899, Studies in the Contraction of Smooth Muscle, *Am. J. Physiol.*, **3,** 26.

1902, Maximal Contraction, Stair-case Contraction, Refractory Period, and Compensatory Pause, *Am. J. Physiol.*, **8,** 213.

Wulzen, R., 1916, The Pituitary Gland: Its Effect on Growth and Fission of Planarian Worms, *J. Biol. Chem*, **25,** No. 3.

Yocom, H. B., 1918, The Neuromotor Apparatus of *Euplotes patella*, *Univ. Calif. Pub. Zoöl.*, **18,**, 337.

Yung, Emile, 1899, La Digestion des Poissons, *Arch. Zoöl. Exp.* (3), **7,** 121.

Zoethout, W. D., 1919, Stimulation by the Outward Diffusion of an Electrolyte from the Irritable Tissue, *Am. J. Physiol.*, **48,** 161.

Zsigmondy, R., 1917, The Chemistry of Colloids, translated by Spear, John Wiley & Sons, Inc., New York.

Zuegler, M., 1910, Influence of Sea Water on Pulsating Vacuole, *Arch. Entwickl. Mech. Organ.*, **29,** 632.

INDEX

A

Abalone, cardio-regulator nerves, 210
Abiogenesis, 534
Absolute refractory period, 470
Absorption, 334*ff*
  carbohydrates and proteins, 340
  coefficient of gas by liquid, 223
  Daphnia, food, 331
  gas by liquid, 223
  kidney tubule, 444
Accelerator nerves, frog, 215
  see cardio-regulator nerves.
Achroödextrin, 30
Achroöglobin, chemical composition, 126
Acrosome, 540
Actinia, oxygen consumption, 237
  respiration, 238
Actinians, digestion, 323
Actinoloba, reaction, 284
Activation of egg, 545
Activators of proenzymes, 268
Adaptation, 75
Addison, 362
Adequate and inadequate stimuli, 474
Adhesiveness, blood cells, 175
Adiposity, allied to activity of pituitary gland, 370
Adjustor neurones, earthworm, 502
Adrenal gland, 367
  relation to sympathetic nervous system, 368
  represented in invertebrates, 367
Adrenalin, role, 368
  Annelids, 368
Adrian, 468, 474, 475
  all-or-nothing law, 470
  gas chamber experiment, 469
Adsorption, interfacial, 24
  Mathew's illustration, 22
  role in catalytic actions, 262
  size of particles of adsorbents, 24
Afferent arc, 472
After discharge, motor nerve and reflex arc, 474
  starfish, 488
Agglutination, 166
  blood cells, 173
  factor in excretion, 433
  factor in fertilization, 546
Alanine, composition, 275
Albumin, chemical composition, 60
Alcyonaria, digestion, 325
  mesenterial filaments, 326
Aldehyde, formation, 90
Alimentary system, 96
  Amphibia, 97
  Annelida, 96
Alimentary system, Aves, 97
  Coelenterates, 96
  Crustacea, 96
  Cyclostoma, 96
  Echinodermata, 96
  Elasmobranchii, 96
  Hemichordata, 96
  Hexopoda, 96
  Mollusca, 96
  Nemathelminthes, 96
  Pisces, 96
  Platyhelminthes, 96
  Porifera, 96
  Protozoa, 96
  Reptilia, 97
  Urochordata, 96
Allmann, 323
All-or-none (nothing) law, 402, 469, 470
Allotropic substances, 7
Alsberg and Clark, 138
  coagulation of blood of Limulus, 166
Amberson, studies on respiration, 237, 238
Amia, rate of digestion, 332, 333
Amino acids, circulating in blood, 343
  classification and formulas, 275
  energy value, 346
  manufactured in body, 343
  needed by protozoa, 297
  products of protein digestion, 272
Ammonia, excreted by sponges, 416
Amoeba, 70
  digestion, 312
  excretory apparatus, 413
  feeding, 311
  movement, 375
  permeability, 68
  reactions of food vacuoles, 312
  structure, 54, 55
  tryptic digestion, 313
Amoebocytes, crayfish, 115
Amoeboid cells, phagocytic action among worms, 432
Amoeboid movement, 374
Amphiaster, 56, 82
Amphibia, alimentary system, 97
  circulating fluid, 100
  circulatory system, 98
  excretory system, 101
  nervous system, 103
  reproductive system, 105
  respiratory system, 99
Amphitrite, 556
  spawning, 542
Amphoteric electrolytes, 33
Amylodextrin, 30
Anabolism, 69, 347

Anaerobic respiration, Ascaris, 232
Andrus, 197
  effect of H′ ion upon heart, 202
Andrus and Carter, H′ ion upon heart, 202
Anemonia, muscle fiber, 390
  oxygen consumption, 237
Anesthetics, effect upon temperature regulation, 355
Animal behavior, vitalistic view, 449
Animal heat, 352
Anion, defined, 15
  effect upon cardiac muscle, 386
  effect upon ciliary action, 383, 386
Anisotropic substance, muscle, 392
Annelida, alimentary system, 96
  circulating fluid, 100
  circulatory system, 97
  excretory system, 101
  nervous system, 102
  reproductive system, 104
  respiratory system, 99
Anodonta, blood, 115
  heart action, 186
  respiration, 239, 240
Anoxybiosis, 240
Antedon, reflexes, 491
Antennary glands, 438
Anthea, nerve plexus, 454, 455
Antirachitic vitamin, 307
Antiscorbutic vitamin, 306
Antisterility vitamin, 307
Antithrombin, 171
Antitoxins, 293
Aortic arches, earthworm, 186
Aplysia, carbon dioxide content of blood, 164
  cardio-regulator nerves, 210, 213
  diagram of vascular system, 179
  oxygen consumption and concentration, 237
Aquatic insects, 243
Arachnida, guanin, 440
Arbacia, 556
  block to fertilization by H′ ion, 550
  effect of H′ ion upon fertilization, 548, 549
  effect of temperature upon cleavage, 564
  fertilization, 546
  hypertonic sea water as agent in artificial fertilization, 556
Area, 133
  blood, 135
Arenicola, cardio-regulator nerves, 208
  haemoglobin, 132
Arginine, composition, 276
Argulus, secretion by unicellular glands, 290
Ariolimax, cardio-regulator nerves, 210, 213
Armadillium, respiration, 241
Armsby, 219
Arnold, digestion in Planaria, 327, 328
Arrhenius, 15, 41
  lunar periodicity of human births, 543
Artemia, blood, 134
Arteries, 183
  elasticity, 186
  functions, 185
Arthropoda, cardio-regulator nerves, 212
  nervous organization, 518
Artificial parthenogenesis, 554*ff*
  agents used, 557
Ascaris, respiration, 232
  striated borders of epithelium cells, 330
Ascidia, blood, 117, 164
Ascidian, Styela, 574
Asellus, respiration, 241
Asexual reproduction, 535
Aspartic acid, composition, 275
Association cells, 524
Astacus, blood corpuscles, 114
  coagulation, 166
  giant fibers, 505
Aster, 56, 82
Asterias, 556
  block to fertilization by H′ ion, 550
  hydrogen ion concentration of pyloric coeca, 284
  parthenogenetic (?), 557
  righting reactions, 489, 490
Asterina, righting reactions, 489
Athrocytes, Echinoderm, 420
  specific action, 420
Atom and electron, 6
  structure, 6
Atomic theory, 6
Atrio-ventricular junction, effect of ligature, 203
Atropine, action upon squid, 457
Atwater, 219
Atwater and Rosa, 348
Augmentor nerves, see cardio-regulator nerves.
  frog, 215
Aurelia, rhythmic pulsation, 485
Auriculo-ventricular node, 207
Automatism and irritability, 380
Aves, alimentary system, 97
  circulating fluid, 100
  circulatory system, 98
  excretory system, 101
  nervous system, 103
  reproductive system, 105
  respiratory system, 99
Avogadro's law, 41, 223
Axial filament, 540
Axiothea, giant cells and fibers, 493
  primitive nervous system, 493

B

Bahnung, 477
Balance experiments, 348
  general results, 349
Balanced actions, 263
Balanced solutions, effect on ciliary action, 383
Balfour's rule, 559
Barbour, 357
Barcroft, mechanics of oxygen diffusion, 259
  spectrum of Arenicola haemoglobin, 132
Barratt, Wakelin, 355
Basal corpuscles, 376, 377, 378
Bases, parthenogenetic agents, 557
Bayliss, 465
  scheme of catalysis, 261
Bedot, excreting individuals of Coelenterate colony, 417

Bee, circulation, 181
lack of enzymes, 331
respiration, 250
vascular apparatus, 180
Beetles, (diving), respiration, 245
Behavior, factors influencing, 447
mechanistic view, 450
organization a factor, 451
vitalistic view, 449
Benedict, 349
Bergmann, 352
Bergmann and Rubner, surface law, 251
Beri-beri, 306
Berlese, 518
Bernard, Claude, 350, 362
Bert, Paul, 245
cardio-regulators, 211
Berthold, amoeboid movement, 374
experiments on testes, 362
Bertrand, nature of enzymes, 266
Bethe, 518
experiments on nervous system of crayfish, 526
Bicarbonates, as buffers, 161
Biedermann, methods of experimentation upon worms, 514
tryptic digestion of invertebrates, 271
Bile salts, parthenogenetic agent, 557
Binet, 518
Biocatalyst, 264
Bioelements, 59
Biogen hypothesis, 61
Biogen molecule, 61
Biogenesis, 534
Bios, 307
Biotic energy, 9, 88
Bipolar cells, 507
Birge and Juday, 125, 224, 225, 237
Bissel, 274
Biuret reaction, 34
Black Sea, oxygen content of water, 230
Blanchard, 134
Blastomeres, isolation, 566
Blatta, excretion, 441
Blood, 107*ff*
buffer action, 160*ff*
coagulation, 165
crayfish, 114
dissociation, fishes, 254
freezing point, 47
functions, 108
gas analysis, 141
invertebrates, chemical composition, 144
Myriapods, 116
oxygen carrying function, 124
quantity in body, 108
reactions, 146
vertebrates, 118
Blood corpuscles, adipoleucocytes, Insecta, 116
area, 118
Ascidia, 112
basophil, Mollusca, 116
Capitella, 120
Cephalopoda, 112
Crustacea, 113
Dytiscus, 117
Blood corpuscles, Echidna, 118
Echinodermata, 111
eosinophil, Mollusca, 116
fat carriers, 114
Glycera, 120
granular, Myriapod, 116
Hydrophilus, 117
irritability, 113
leucocytes, Insecta, 116
Llama, 118
Nemerteans, 111
number, 118
oat-shaped, 116
oenocytoids, Insecta, 116
Oligochaetae, 112
phagocytes, Insecta, 116
Phoronis, 120
Polychaetae, 112
proleucocytes, Insecta, 116
shape, 119
Sipunculid worms, 112
sizes, 118
Solen, 120
spherule cells, 116
Blood pigments, as wastes, 120
invertebrates, 120
Blood platelets, 122
Blood pressure, 185
role of adrenalin, 368
Blood proteins, as weak acids, 114
Blood salts, relative concentration, 151, 152, 153
Blood serum, medium for ciliary action, 381
Blood sinuses, 185
Blood sugar, 350
Bodensee, oxygen content, 230
Body fluids, concentration and osmotic pressure, 146
correspondence to sea water, 146
Body fluids and sea water, correspondence in H′ concentration, 158
correspondence in osmotic pressure, 146
correspondence in relative concentration of salts, 151
correspondence in temperature, 157
Body fluids, freezing points, 142, 143
invertebrates, analyses, 145
Body mass and body surface, 357
Body surface, DuBois' formula, 358
Meeh's formula, 357
relation to cell surface, 358
relation to respiration, 251
Body temperature, daily variations, 355
lower animals, 353
Bombyx, respiration, 243
Bone, 292
Borup-Besancz and Will, 438
Botazzi, cardio-regulators, 211
freezing point of urine, 429
Bovard, 514
rate of transmission in earthworm, 513
Bowditch, all-or-none law in frog heart, 402
Bowman, excretion by kidney, 442
Boyle's Law, 41, 223
Brachynotus, effect of salts upon heart action, 198

Brain, bee, figured, 532
Brain, freezing point, 47
Branchiopods, blood, 134
Branchipus, brain, 519
central nervous system, 518–521
haemoglobin, 134
nerve cells and fiber tracts, 519–521
number of nerve cells in ganglia, 521
ventral ganglia, 519, 520
Brandt, 311
Brownian movement, 20
protoplasmic granules, 56
Brown-Sequard, 362
Brucke, fat absorption, 336
Bruntz, 117
Buchner, 285
isolation of zymase, 264
Budding, 535
Budington, vascular nerves of Venus, 209
Buffers, 160
blood, 161
chemical reactions, 163
laws of action, 162
role of blood cells, 163
role of blood proteins, 160
role in carbon dioxide transport, 160
Bunge, 231
Burker, 135
Bütschli, 309
Butyric acid, agent in artificial parthenogenesis, 555
Byssus, 292

C

Caffeine, action upon invertebrate nerve cells, 457
Calcium carbonate, excreted by Arachnida, 440
Calcium, diffusion, 50
effect upon cardiac muscle, 199, 386
effect upon ciliary action, 383, 386
effect upon separation of blastomeres, 567
essential for skeleton formation, 572
role in metabolism, 171
Calcium metabolism, and parathyroid glands, 371
Calcium oxalate, excreted by Arachnida, 440
Calcium phosphate, excreted by protozoa, 415
Calliburces, ciliary action in frog, 384
Callinectes, respiration and oxygen tension of water, 238
Camphor, action upon nerve cells of squid, 457
effect upon nervous system of shrimp, 457
Canandaigua Lake, oxygen content, 227
Cancer, coagulation of blood, 166
gills, 242
Capillaries, 183
contraction due to Rouget cells, 188
distension correlated with work, 188
function, 187
nerve control, 190
number in various animals, 188
organs for diffusion, 185
permeable by proteins, 188
phyletic history, 178
tonus of walls, 189
value to organism, 188
Capitella, haemoglobin, 131
Carbohydrates, 30
inadequate for protein building, 347
metabolism, 349
those most used for food, 350
Carbon cycle, 300
Carbon dioxide, a condition for life, 87
amount on the earth, 300
carried by serum and cells, 162
content in blood of marine invertebrates, 164
effect upon development, 571, 572
effect upon dissociation of haemoglobin, 129
effects upon Carrassius, 253
effects upon fishes, 254
elimination by contractile vacuole, 413
how carried in blood, 160*ff*
method of determination, 234
normal product of muscle, 398
parthenogenetic agent, 556
produced by frogs in nitrogen, 231
produced by nerve, 464, 465, 466
production in muscle due to direct combustion, 407
production in various animals, 236
role in staircase contraction, 400
Carbon equilibrium, 347
Carbon-monoxide-haemoglobin, 127
Carcinus, 526
coagulation, 166
gills, 242
Cardiac muscle, compared with cilia, 386
Mollusca, 390
Cardio-regulator nerves, Abalone, 210
Aplysia, 210, 213
Ariolimax, 210, 213
Cryptochiton, 210, 211
grasshopper, 213
Haliotis, 210, 212
invertebrates, 207
leech, 209
Loligo, 211, 214
Mya, 210, 211
Mytilus, 210, 211
Palinurus, 212, 214
Pecten, 210
Polyphemus moth, 213
Tapes, 210
Venus, 210
vertebrates, 214*ff*
Cardium norwegicum, blood, 116
Carlson, cardio-regulator nerves, 205, 207, 210*ff*
Carlson and Jenkins, rate of nervous transmission in worms, 516
Carrassius, effects of carbon dioxide, 253
Carter, 197
Carter, nutrition of sponges, 321
Cartilage, 292
Cash, 335
Cassiopea, rhythmical pulsation, 485
Catabolism, 69, 347
Catalysis, Bayliss' scheme, 261
Catalysts, characteristics, 262
enzymes, 263
mode of action, 262
Catalytic actions, 261

Catastomus, muscle structure, 392
Cation, defined, 15
  effects on cardiac muscle, 386
  effects on ciliary action, 386
Celakowsky, 310
Cell, adaptations for absorption, 79
  adaptations for movement, 78
  adaptations for reception and conduction of impulses, 79
  adaptations for reproduction, 80
  adaptations for secretion, 79
  differentiation, 75
  division, 56, 80
  endogenous division, 535
  enucleated, powers, 73
  irritability, ciliated, 380
  mechanism of division, 537
  modifications, 77
  structure, 72
Cell fibrin, 166
Cell membrane, character, 74
  diffusion through, 74
  function, 74
Cells as transformers, 10
Cellulose, 30, 292
Central nervous system, earthworm, 500
  worms, 494
Centralization, bee, 532
  nervous elements, 473
  nervous elements of worms, 491
Centrechinus, periodicity of spawning, 541
Centrosome, 82
Centrospheres, 83
Cephalization, bee, 532
  Nereis, 493
  nervous elements, 473
Cephalopoda, cardio-regulator nerves, 211
  excretory organs, 435
  haemocyanin, 137
Cephlain protein, 171
Ceradot, uric acid in Malpighian tubules, 440
Cerambyx, excretion, 441
Cercariaeium, muscle cell, 390
Cerebral ganglia, function in crayfish, 526
  function in earthworm, 512
Cerfontaine, structure of ganglia of lumbricus, 509
Chaetogaster, reaction of alimentary tract, 330
Chaetopterus, 556
  block to fertilization by H′ ion, 550
Chambers and Dawson, undulating membranes, 387
Chambers, observations on Brownian movement, 56
  structure of protoplasm, 53–56
Chance of collision, phagocytosis, 174
Chapeaux, intracellular digestion, coelenterates, 324
Charles' law, 223
Chaussier, uric acid in silk worms, 442
Cheirocephalus, haemoglobin, 134
Chemical stimulation, Yungia, 511
Chemoreceptors, 462
Chemotaxis, phagocytic cells, 437
Chitin, 292
  excretophore cells, 432
Chironomous, haemoglobin, 133
  respiratory habit, 243
Chiton, excretory organ, 436
  spawning, 542
Chlorocruorin, chemical composition, 126
Chlorogogue cells, 431
Chlorophyll and oxygen content of water, 229, 230
Choanocytes, sponges, 316
Chondrin, 292
Chorda tympani nerve in secretion, 290
Chromaffin cells, leech, 209
  secrete adrenalin, 369
Chromatin, cell division, 82
Chromoproteins, 125
Chromosome map, 577
Chromosomes, 538, 539, 576
  protein nature, 72
Chun, 325
Church, ionic content of the sea, 151
Cilia, 78, 376
  compared with cardiac muscle, 386
  fatigue, 380, 381
  role in excretion, 424, 436
Ciliary action, behavior of cell colloids, 383
  effect of osmotic pressure, 386
  effect of oxygen, 385
  effect of temperature, 384, 385
  effects of cations, 381, 382
  effects of H′ ion, 382, 383, 384
  fatigue, 381
  reversal, 387
  stoppage due to lack of conversion of chemical into potential mechanical energy, 384
Ciliary mechanism, 376
Ciliary rootlets, 379
Ciliated cells, irritability, 380
  structure, 377
  work done, 381
Cilium, cytoplasmic strand, 377
  structure, 377, 378, 379
Circulating fluid, Amphibia, 100
  Annelida, 100
  Aves, 100
  Coelenterata, 100
  Crustacea, 100
  Cyclostoma, 100
  Echinodermata, 100
  Elasmobranchii, 100
  Hemichordata, 100
  Hexopoda, 100
  Mammalia, 100
  Mollusca, 100
  Nemathelminthes, 100
  Pisces, 100
  Platyhelminthes, 100
  Porifera, 100
  Protozoa, 100
  Reptilia, 100
  Urochordata, 100
Circulation, Coelenterates, 325
  Crustacea, 179, 180
  discovery, 184
  Mollusca, 179
  Porifera, 177
  Protozoa, 176

Circulatory mechanisms, 176
Amphibia, 98
Annelida, 97
need for, 176
Circulatory system, Aplysia, 179, 180
Aves, 98
Coelenterata, 97
Crustacea, 97
Cyclostoma, 98
Echinodermata, 97
Elasmobranchii, 98
Hemichordata, 98
Hexopoda, 97
lobster, 180
lung fish, 182
Mammalia, 98, 182
Mollusca, 97
Nemathelminthes, 97
Palaemon, 179, 180
Pisces, 98
Platyhelminthes, 97
Porifera, 97
Protozoa, 97
Reptilia, 98
salamander, 182
selachian, 182
Urochordata, 98
Circum-oesophageal connectives, effects of cutting, crayfish, 527
Circus movements, crayfish, 527
limulus, 529
Cirri, 377
Clark, 197
Classification of animals, based on blood color, 112
Clavalla, haemoglobin, 134
Cleavage, 536
Balfour's rule, 559
effect of temperature, 564
Hertwig's rule, 559
Sach's rule, 559
types, 559
Cleveland, experiments upon Termites, 235
nutrition of Termites, 298
symbiosis of Termites and Protozoa, 299
Clonus, 401
Clostridium, 302
Clowes, 57
Clowes and Smith, effect on pH on fertilization of eggs, 549, 550
Clymene, giant cells and fibers, 493
primitive nervous system, 493
Coagulation, blood, 165
blood of Echinodermata, 165
frog's blood, 169
Limulus, 165
mammalian blood, 169
Mollusc (Cardium), 165
reversal of phase, 170
zoological significance of types, 168
Cockroach, excretion, 441
Coelenterata, alimentary system, 96
circulating fluid, 100
circulatory system, 97
enzyme activities, 281
excretion, 415, 416
Coelenterata, excretory system, 100
muscular elements, 390
nervous mechanism, 482–486
nervous system, 102
nutrition, 322*ff*
reproductive system, 104
respiratory system, 98
tryptic digestion, 323
Coelomic epithelium, as excretory organ, 435
Coelomic fluid, Echinodermata, 110
Coenzymes, 268
Cohesion, 24
in adsorption, 23
Cohn, 549
effect of H′ ion on respiration of spermatozoa, 258
role of H′ ion in activity of sperm, 260
Collagen, 292
Collip, 47, 164
Colloid, 17
Colloidal behavior, 38
cell division, 81, 82
Colloidal condition, essential for life, 87
Colloidal particles, size, 26, 27
Colloidal sol, 17
Colloidal solutions, as transformers, 10, 11
Collodion membranes, 28
Colloids, active adsorbents, 23
combinations with other substances, 25
compared with crystalloids, 17
dynamic in behavior, 88
effective in synthesis, 90
food substances, 270
instability, 62
molecular weight, 28
osmotic pressure, 26
role in physiology, 29
Colpidium, experiments upon culture medium, 297
respiration, 235
Composition of medium, effect on development, 544
Compound substances, 8
Concentration point, 207
Concentric corpuscles of Hassall, 365
Conchiolin, 292
Concrement glands, Clystoma, 437
Condensation, nervous elements, 473
Conduction, effect of H′ ion on heart, 200
nerve trunk and reflex arc compared, 473, 474
rate, 472
Conductivity, protoplasmic, 67
starfish, 488
Conesus Lake, oxygen content, 225
Congo red, use in digestion studies, 283
Conklin, formative substances, 574
Connective tissue threads, 292
Consciousness, fundamental property of protoplasm, 70
Conservation of energy, law, 12
Conservation of matter, law, 12
Contact digestion, 324
Contact receptors, 462
Continuity, law, 11
protoplasmic, 71

Contractile vacuole, carbon dioxide elimination, 413
effect of temperature upon contraction, 414
excretory organ, 413
rate of contraction, 414
reaction of content, 415
sol-gel reversibility, 413
substances eliminated, 414
variations in permanence, 413
Contractility, 67
effect of fatigue, 403
Contraction, effect of temperature, 398
isometric, 396
isotonic, 396
related to strength of stimulus, 468
Contracture, muscle, 403, 404
Convergence of impulses, 476
Convoluta, spawning, 542
Copepods, haemoglobin, 134
Copper, component of haemocyanin, 137
in crustacean and molluscan bloods, 140
relation to oxygen carrying power, 140
Corallin, 293
Corpus striatum, role in heat production, 357
Corpuscles, number in frog, 168
Corti, 309
Cortical reaction in fertilization and artificial parthenogenesis, 556
Corydalis, 518
motor cells and fibers, 524
muscle, 393
nervous system, 521, 525
sensory fiber tracts, 524
sympathetic nervous system, 523
Cotte, excreta of sponges, 416
Crab, effect of salts upon heart action, 198
gill, 242
respiration, 242
Crawford, 359
Crayfish, blood, 114
central nervous system, 525
experiments upon nervous system, 526
Creatin, excreted by molluscs, 436
Creatinine, excreted by molluscs, 436
Creation, 9
Critical periods of development, 560
Crossed extension reflex, 478
Crozier and Moore, effect of tension on orientation, 517
Crustacea, alimentary system, 96
blood corpuscles, 113
circulating fluid, 100
circulatory system, 97
coagulation of blood, 166
digestion, 331
double circulation, 134
excretion, 438
excretory system, 101
nervous system, 102
reproductive system, 104
respiratory system, 99
types of muscle, 391
Cryoscopy, 46
Cryptochiton, cardio-regulatory nerves, 210, 211
Crystalline style, a digestive structure, 332
factor in respiration, 240
functions, 332
Cucumaria, nutrition, 317
Cuenot, 133, 429
chlorogogue tissue, 431
significance of haemoglobin, 135
Culex, respiratory habit, 243
Cunningham, 338
Cushny, theory of excretion, 443
Cuticle of ciliated cells, 378
Cuvier, 112
Cyanide, effect on cardiac muscle, 386
effect on ciliary action, 386
Cyanogen compounds, 61
Cycles in nature, 299
Cyclic changes, 63
Cyclops, lack of oxygen, 232
Cyclostomata, alimentary system, 96
circulating fluid, 100
circulatory system, 98
excretory system, 101
nervous system, 103
reproductive system, 105
respiratory system, 99
Cystine, composition, 275
Cytolysis, 555
Cytolysis of egg, 551
Cytoplasm, 55
structure and composition, 74
Cytoplasmic strands of cilium, 377

D

Dactylometra, rhythmical pulsations, 485
Dahlgren and Kepner, muscle structure, 391–395
Dale and Clark, 200
Dale and Thacker, 200
Dalton, atomic theory, 6
Dalton's law of partial pressures in gas exchange of swim bladder, 256
Damant, respiration of crabs, 242
Daphnia, absorption of food, 331
blood corpuscles, 113
haemoglobin, 134
mid-gut, 331
Davy, 440
Dawson, 516
nerve cells of nerve rings, earthworm, 501
Deaminization, 347
DeBordeu, 361
DeBruyne, 115
Decerebrate animal, 478
rigidity, 478
Decrement, 469
region, 470
Definite proportions, law, 11
Delage, 554
theory of fertilization, 550, 551
Dendrites, 458
Dentine, 292
Development, a series of reactions, 559, 560
critical periods, 560
effect of carbon dioxide, 571
effect of combinations of salts, 573

Development, effect of external factors, 561–573
effect of osmotic pressure, 561
effect of oxygen, 570
effect of pressure, 561
effect of potassium, 572
effect of sodium and calcium, 572
effect of sulphion, 573
effect of temperature, 562-566
effect of variations in chemical environment, 568
evolution of care of eggs, 557, 558
factors involved, 543
hazards involved, 557, 558
internal factors, 574
isolation of blastomeres, 566
vascular system, 178
Dextrin, 30
Dextrose, effect on ciliary action, 386
Dialysis, applications in physiology, 50
Diapedesis, a factor in excretion among worms, 433
Diastase, discovery, 263
sponges, 322
Diemyctylus, body temperature, 195
fat absorption, 335
Differentiation, 75
and growth of germ cells, 536
directions, 77
Diffuse spreading of nerve impulses, Coelenterates, 482
Diffuse transmission, nerve net, 456
Diffusion, 39
oxygen in water, 229
oxygen through respiratory epithelium, 258
relation to respiration, 238
role in gas exchange of swim bladder, 326
Diffusion pressure, 39
role in osmosis, 43
Digestion, Alcyonaria, 326
Coelenterata and worms compared, 327
contact, 324
Crustacea, 331
effects of parasitism, 329
extra cellular, 270
factor in excretion, 433
fat, 276
intracellular, 270, 321
mesenterial filaments in Coelenterata, 322, 324
Planaria, 327
Platyhelminthes, 326
Protozoa, 268
rate in Amia, 332, 333
starch, 277
work of, 270
worms, 326
Digestive epithelium, glandular and absorptive, 330
Digestive vacuoles, reaction in protozoa, 268
Diplopods, homostrophic reflex, 517
Discomedusae, excretory organs, 417
Dissociation, 15
blood of fishes, 254
degree, 16
Dissolved matters as food, 318, 319
Division of labor, 75
Dixon, 310
Dominance (physiological), earthworm, 493
Nereis, 493
relation to distribution of receptors, 495
Donnan, 38
equilibrium, 35
Drew, 165
Driesch, effect of temperature in producing evaginate gut, 565
Drosophila, 576
Drury, 122
DuBary, 310
Dubrunfaut, 263
Duclaux, 244
Dujardin, 126
Durham, excretion of ink by Echinoderm egg, 419
Dyes, 293
Dysek, 256
Dytiscus, blood, 117
blood corpuscles, 117
respiration, 245

E

Earthworm, chain of elements involved in conduction, 508
choice of food, 496
dorsal blood vessel, 186
nephridium, 427, 428
nerve plexus systems, 497, 498
nervous system, 497*ff*
nervous system, anterior end, 499
photoreceptors, 495, 496
rate of nervous transmission, 515
receptors, 495
respiration, 239
structure of ganglion, 506
Echidna, blood corpuscles, 118
Echinarachnius, hypertonic sea water in artificial parthenogenesis, 556
Echinodermata, alimentary system, 96
circulating fluid, 100
circulatory system, 97
enzyme action, 281
excretion, 417
excretory system, 100
haemoglobin, 111
internal media, 110, 111
methods of excretion, 417
nervous system, 102, 486
reproductive system, 104
respiratory system, 99
storage excretion, 420
urea, 417
Echinus, excretion, 420
hydrogen ion concentration of digestive capsules, 284
temperature coefficient of movements of mouth parts, 284
Ectoenzymes, 264
Ectoneural nervous system, Echinodermata, 486
Ectoplasm, 74, 375
Edkins, 292
Egg and sperm shedding, 541
Egg cell, 80

Egg cell, decrease in diameter upon fertilization, 547
diversity of structure and composition, 537
highly specialized, 536
irritability, 536, 537
mechanism of cell division, 537
part in heredity, 537
rate of oxidation, 258
specificity, 537
stores of energy, 537
Elasmobranchii, alimentary system, 96
circulating fluid, 100
circulatory system, 98
excretory system, 101
nervous system, 103
reproductive system, 105
respiratory system, 99
Elastic tube system, 190, 191
Elasticity, muscle, 395
Electrical changes involved in heart action, 200
Electrolytes, amphoteric, 33
Electron, mass, 6
size, 6
Eledone, haemocyanine crystals, 138
oxygen consumption and concentration, 237
Emulsion, 14, 281
and emulsoids, physical properties, 20
as loci for chemical combinations, 25
Emulsoids, behavior, 21
properties, 21
Endocrine glands and secretions, 361
methods of experimentation, 364
nature, 362
role, 364
Endoderm, an excretory tissue, 416
Endoenzymes, 264
Endogenous cell division, 535
Endothelium, vascular wall, 178
Energy and energetics, 9
Energy, biotic, 9
expenditure during fertilization and cleavage, 554
transformation, 10, 85
transformers, 10
Englemann, 311
Entoneural nervous system, Echinodermata, 478
Entoplasm, 375
Entozoa, need for oxygen, 231
Environment, role in development, 559, 560
Enzymes, 293
action upon carbohydrates, 277
catalysts, 263
characteristics, 265
chemical nature, 265
classification, 278, 279
defined, 264
distribution in animal kingdom, 279, 280
effect of heat, 266
evolution, 285
general properties, 266
invertebrates, 281
optimum temperature, 267
role of H′ ion, 267
significance of reversible action, 276
specificity of action, 274
Enzymes, sponges, 322
synthetic actions, 274
universal occurrence, 285
Eosinophil cells, fish, 122
Epilimnion, 226
Erepsin, 267
digestion, 272
Erythrocytes, 118
shape, 119
Erythrodextrin, 30
Ethereal vibrations, range, 460, 461
Eupagurus, excretion, 439
Euplotes, 452
rate of operation of contractile vacuole, 414
Evans and Bishop, vitamin E, 307
Evolution, use of carbohydrates a sign, 349
Excitable tissue, elementary functions, 463, 464
Excreta, Echinodermata, not guanin, or xanthin, 420, 421
Excretion, 422*ff*
absorption by kidney tubule, 444
alimentary epithelium, 418
antennary gland, 438
character of liquid, 443
Coelenterata, 415, 416
correlated with vascular apparatus, 422
Crustacea, 438
crystal or granule formation in protozoa, 415
Cushny's theory, 443
diffusion, 417, 418
diffusion due to pressure difference, 421
effect of hibernation, 437
elimination and resorption among worms, 421
excretophore tissues, 437
factors influencing permeability of kidney cells, 443
fat bodies, 440
forms of filtering mechanisms, 422
glandular, 417
granular, among worms, 429
holocrine, 429, 430
Insecta, 440*ff*
intestinal epithelium, 439
intracellular, 417
intracellular, Echinoderm, 419
localized, Coelenterata, 416
merocrine, 429, 430
metazoa, 415
mode, Crustacea, 439
Mollusca, 435*ff*
oral branchii, 418
pericardial gland, Mollusca, 437
physical process, 422
process in Mollusca, 436
Protozoa, 413
rate in worms, 428
resorption of water among Mollusca, 436
respiratory papillae, 418
respiratory tree, 418
role of amoeboid cells, sponges, Coelenterates, 416
role of endoderm, Coelenterates, 416
role of genital organs, Echinodermata, 419
role of heart bodies, 431
role of membrane, 443

Excretion, role of pressure, 443
selective diffusion, 422
shell gland, 438
skin, 439
sponges, 415, 416
stains, by insects, 442
storage, 416, 417, 420
superficial-intracellular, among sponges, 416
types of filtering mechanisms, 423
worms, 421
Excretophore cells, chemical nature of content, 432
Excretophore substances, fate, 422
nature, 432
Excretophore tissues, Mollusca, 437
Excretophores, 430
extravasal, 431
intravasal, 430
Excretory organs, functions, 412
fundamental structure, 421
Insecta, 441
lacking in some endoparasites, 413
Medusae, 417
Mollusca, 435
worms, 423
Excretory system, Amphibia, 101
Annelida, 101
Aves, 101
Coelenterata, 100
Crustacea, 101
Cyclostoma, 101
Echinodermata, 100
Elasmobranchii, 101
Hemichordata, 101
Hexopoda, 101
Mammalia, 101
Mollusca, 101
Nemathelminthes, 100
Pisces, 101
Platyhelminthes, 100
Porifera, 100
Protozoa, 100
Reptilia, 101
Urochordata, 101
Exhaustion, nervous, 466
Exosmosis, from eggs during artificial parthenogenesis, 556
Explosive corpuscles, 114
in coagulation, 166
Extensibility, muscle, 395
External factors of development, 544
Exteroceptors, 462
Extravasal excretophores, 431

F

Facilitation, 477
Factors influencing behavior, 447
False aquatics, 242
Fat, 31, 293
absorption as soaps, 336
use in body, 352
Fat absorption, Daphnia, 331
figures, 336, 337, 338
historical statement, 335
Fat absorption, possible methods, 334
Fat digestion, 276
Planaria, 327
Fat transport, by blood corpuscles, 114
Fatigue, causal agents, 404
cilia, 381
frog muscle curve, 403
influence of lack of oxygen, 403, 404
muscle, 401
nerve trunk and reflex arc, 475
nervous, 466
role of lactic and phosphoric acids, 401, 403
Fatty acids, parthenogenetic agents, 557
Fenn, studies on phagocytosis, 172*ff*
Fermentation, yeast, 264
Ferments, 263
organized and unorganized, 263
Ferric salts, excreted by Mollusca, 436
Fertilization, 536, 545*ff*
agglutination of sperm involved, 546
block by H′ ion, 549, 550
decrease in diameter of egg, 547
effect of H′ ion, 547
effect on oxidation processes in egg, 258
fertilizin involved, 552
heat production, 553, 554
initiates cell division, 555
latent period, 546
Fertilization membrane, 545, 551
elevation, 547
indicator of fertilization, 555
number of spermatozoa required, 552
optimum H′ ion concentration, 548, 549
oxidation process involved, 547
penetration of spermatozoon, 546
permeability of membrane, 547
physical change involved, 546, 551
reaction, 546
role in biparental inheritance, 555
role of lysin, 551
roles of activity of sperm and of egg protoplasm, 545, 546
sterilization a factor, 546
theories, 550*ff*
Fibrillae, muscle, 391
Fibrin crystals, 170
Fibrin, Limulus, 165, 166
photograph of human, 170
Fibrin threads, 170
Fibrinogen, 170
Filtering mechanism, 423
Final pathway, 472
Fischer, 554
Fishes, oxygen concentration and consumption, 237
respiration, 251
Fission, 535
Flagella, accessory to nephridia, 426
Flagellum, 78
Flame cell, 424
functions, 428
Flame chamber, 424
Flatworm, excretory apparatus, 423
Fletcher and Hopkins, 406
lactic acid and fatigue, 404

Fluid medium, 142
ancestry, 142
Foetal membrane formation, 536
Fol, fertilization of egg, 546
Food supply, conditioned by environment, 320
Food taking, Infusoria, 314
sponges, 320
Food vacuoles, dimensions, 315
number, 315
reactions, 310
Food stuffs, complexity, 296
Foods, average composition, 59
brief classification, 303
colloidal character, 270
demanded by protozoa, 271, 297
general characters, 303
nature of solution in alimentary tract, 342
use in body, 342
Forbes, 474, 475, 476
comparison of heart muscle, skeletal muscle, and nerve fiber, 471, 472
crossed extension reflex, 478
rate of motor impulses, 468
Forces acting upon organism, 448
Form and function, 75
Form, changes, 85
Formative substances, 574
Fortuyn, organization of ganglion of earthworm, 506, 507
Foster, M., 61
Fredericq, 322, 323
cardio-regulator nerves, 212
haemocyanin, 136
Freezing points, bloods and body fluids, 143
determination, 46
relation to osmotic pressure, 44
Friedlander, 506
experiments upon earthworms, 513, 514
Friedmann, digestion among Coelenterates, 325
Friend, H., 239
Frog, accelerator nerve, 215
action of spiral valve, 186
arterial arches, 186
augmentor nerve, 215
blood, 168
heart, effects of H′ ion concentration, 201
rate of digestion, 332, 333
Fry, 123
cardio-regulators, 211
Fuchs, 212
Fuld and Spiro, theory of coagulation, 172
Fuligo, 310
Function and form, 75
Functions, cellular, 75
general, 75
special, 75
Fundulus, effects of oxygen on development, 571
effects of temperature upon development, 565
rate of oxygen consumption during development, 258

G

Gametes, 536
Gammarus, section of nephridial canal, 438
Ganglion cells, 502
Ganglion, minute structure, earthworm, 506
Gardner and Leetham, respiration of trout, 252
Garrey, 554
Limulus heart, 194, 205
rate of motor impulses, 468
Garrey and Moore, reciprocal innervation, earthworm, 514
Gas chamber experiment, 469
Gas laws, 222, 223
Gaskell, 368
vascular nerves, leech, 209
Gastrin, 292
Gastrocnemius, twitch compared with that os soleus, 411
Gastro-vascular system, Coelenterata, 325
Gay-Lussac's Law, 41
Gel, 15
Gelatine, conductivity, 37
isoelectric point, 37
osmotic pressure, 37
physical properties, 37
swelling, 37
viscosity, 37
Gelation, cell division, 83
Gemmation, 535
General functions, 75
Geodia, pepsin, 322
Germ cells, development cycles, 538
hormones, 538
Insecta, structure, 56
physico-chemical systems, 538
Germ nuclei, fusion, 546
Giant fibers, 502
Axiothea, 493
Cambarus, 505
connections with neuropile, 505
crayfish, 527
diagonal septa as synaptic membranes, 505
earthworm, 503, 504
multiple synapses, 505
Palaemonetes, 505
photograph, preparation by Hess, 504
photograph, preparation by Stough, 503
Gills, 222
Cancer, 242
Carcinus, 242
ciliated action, 380
crab, 242
Portunus, 242
structure, 240
Gland cells, functions in Coelenterata, 325
Glands, 265, 287
changes in cells during secretion, 288
fundamental structure, 418
fundamental type of structure, 288
types of work done, 288
Glomerulus, 425
function according to Bowman, 442
Glutamic acid, composition, 275
Glycera, 131
Glycine, formula, 275
Glycogen, 30
Arthropods, 351
disappearance under anaerobic conditions, 240

Glycogen, formation and use, 350
Mollusca, 351
occurrence among lower animals, 350
Protozoa, 351
work on, a stimulus for study of ductless glands, 362
worms, 351
Glycogenase, 350
Golgi, 457
Gonionemus, nervous system, 483, 484
Gorup-Besanez, 440
Göthlin, nature of nerve action, 467
Graham, 38, 88
Graphic method, 388
Grasshopper, cardio-regulator nerves, 213
functions of spiracles, 248
respiration, 246
Grave and Schmidt, cilium structure, 378, 379, 380
Gravity, effect on development, 544
Gray, effect of oxygen on ciliary action, 385
studies on cilia, 382
Greene, 335, 554
Greenwood, 313
Greenwood and Meissner, 313
Greenwood and Saunders, 313
Griffeths, 126, 132, 138
Grobben, chlorogogue tissue, 431
Growth, 70
Guanin, Coelenterata, 416
excreted by Arachnids, 440
excreted by Crustacea, 439
found in excretophore cells, 432
not found in echinoderm excreta, 420
Gudernatsch, 367
Gums, 31
Gurwitch, 285

H

Haeckel, 317, 321, 438
Haematin, chemical composition, 126, 127
Haematoporphyrin, 126, 132
Haemerythrin, chemical composition, 126
Haemin, crystals, 131
Haemocyanin, 136
analysis, 138
crystallization, 138
distribution in animal kingdom, 136
importance of copper, 139
Octopus, 136
oxygen carrying function, 139
properties, 137
spectrum, 138
Haemocyanins, are there many?, 140
Haemoglobin, amount in body, 130
carbon dioxide carrying function, 135
carried in corpuscles, 255
chemical composition, 126
crystals, 130, 131
Echinoderms, 111, 133
factories, of worms, 431
Mollusca, 133
oxygen combining power, 130
physical chemistry, 127
reduced, and reducing agents, 128
Haemoglobin, relation to other blood pigments, 120
significance in lower forms, 135, 238
spectroscopic examination, 127, 128
transport in body, 129
variations in crystallization, 131
worms, 112, 131
Haemolymph, 107
Haldane, diffusion of oxygen through respiratory epithelium, 259
osmotic pressure, 42
Haliotis, cardio-regulator nerves, 210, 212
Hall, F. G., gases in swim bladder, 255
Haller, 196, 322, 506, 518
Hardy, 113, 114, 115, 166,
studies on protoplasm, 53
Harless, 137
Harless and Bibra, 137
Hartog, 310
Harvey, circulation of blood, 184
Hawk, absorption of fats, 338
variations in water ingestion, 304
Hazards of development, evasions, 557*ff*
Head (spermatozoon), 540
Heart, 184
bee, 180
chick embryo, 203
crab, 192, 196
crayfish, 196
freezing point, 47
frog, 186
Limulus, 205
need for oxygen supply, 192
nerve cells, 196
physiology, 192
primary function, 185
Heart action, Anodonta, 186
due to fluid bathing tissues, 197
effect of oxygen on crab, Fig. 27, 193
effect of temperature, 194
Heart beat, myogenic theory, 197
neurogenic theory, 204
origin, 196
record, frog, 187
Heart block, chick embryo, 202
Heart bodies, Annelida, 431
excretory function, 431
Heart valves, frog, 187
Heat centers, heat nerves, 356
Heat, factor conditioning life, 86
liberated by muscle, 408
Heat loss, factors regulating, 358
Heat production, absolute value in tetanus, 410
and heat loss, 355
during fertilization and cleavage, 553
during muscle contraction, 407
during muscle relaxation, 407
factors influencing, 356
independent of frequency of excitation, 409, 410
isotonic and isometric contractions, 408
muscle, 405, 406
proportional to length of fiber, 408
related to lactic acid production by muscle, 408

Heat production, terrapin heart, 215
tetanus and chloroform poisoning, 408
Heat rigor, muscle, 408
Heidenhain, 290
essentials of neurone theory, 457
kidney action, 442
Heilbrunn, 554
fertilization due change in physical properties of egg, 551
structure of protoplasm, 58
Heliozoa, food habits, 308
Helix, cardio-regulator nerves, 211
haemocyanin, 137
section of kidney, 437
Helmholtz, rate of transmission of nerve impulse, 464
Hemichordata, alimentary system, 96
circulating fluid, 100
circulatory system, 98
excretory system, 101
nervous system, 102
reproductive system, 104
respiratory system, 99
Hemlock Lake, oxygen content, 226
Hemmeter, 310
Henderson, carbon dioxide transport in blood, 135
laws of buffer action, 162
Henle, 361
Henri and Lalou, permeability of membranes, Echinodermata, 418
Henry's law, 223
Henze, 138, 237
Herbst, 567
formula for artificial sea water, 569
Hermann, inogen molecule, 406
Hermella, blood pigment, 127
Herrick, nerve elements involved in conduction, earthworm, 508
nerve elements involved in locomotion, 509, 510
Hertwig, 322, 554
nerve cells of Anthea, 455
Hertwig's rule, 559
Hess, subepidermal nerve plexus of earthworm, 498
Hewson, 113
Hexopoda, alimentary system, 96
circulating fluid, 100
circulatory system, 97
excretory system, 101
nervous system, 102
reproductive system, 104
respiratory system, 99
Hexosephosphate, heart muscle, 192
muscle, 398 407
Hibernation, 437
respiration of silk worm eggs, 244
Hill, 406
heat production by nerve, 464
role of lactic acid in muscle, 408
Hilton, 518
Hippuric acid, excreted by Mollusca, 436
Hircinia, pepsin, 322
Hirudo, chromaffin cells, 369
vascular nerves, 209
Histidine, composition, 276
Holothurian, excretion, 420
Homarus, blood pigment, 137
carbon dioxide production, 466
respiration, 237
Homoiothermous animals, 352, 354
Homoiothermous condition, why desirable?, 355
Homostrophic reflex, diplopods, 517
worms, 517
Honey bee, nervous system 531–533
Hooke, Robert, respiration, 219
Hooker, 190
Hormones, 293*ff*
egg cells, 538
role in development, 575
Howell, 197, 206
coagulation in Limulus, 166
coagulation of mammalian blood, 170
theory of coagulation, 171
Howland, excretory apparatus of Amoeba and Paramoecium, 413
Hufner, 230
Hunger, receptors, 462
Hunter, John, 352
types of voluntary muscle fibers, 411
Hunter and Royle, 411
Huss, respiration in Cyclops, 232
Huxley, 438
Hyaloplasm, 375
Hyde, experiments on nervous system of Limulus, 529
nervous system of Gonionemus, 483, 484
Hydra, nerve net, 453, 454
Hydrates, 48
Hydrogen ion, concentration in body fluids, 159
concentration in physiological saline solutions, 200
concentration in pyloric coeca of Asterias, 284
conditioning life, 88
conversion table pH to Hc, 159
effect on ciliary action, 382, 386
effect on contraction of parts of heart, 200, 201
effect on development, 544
effect upon fertilization, 547
favorable for digestion, 273
potential hydrogen values, 159
relation to muscular fatigue, 401
relation to phagocytosis, 173
relation to respiration, 260
relation to respiration in fishes, 253
role in staircase contraction of muscle, 400
significance, in blood, 260
Hydrogen peroxide, heart action, 192
Hydrolymph, 107
Hydrolysis, 265
Hydromedusae, excretory organs, 47
Hydrophilus, blood corpuscles, 117
Hydrostatic pressure, a measure of osmotic pressure, 42
Hyman, respiration in Planaria, 239
Hymenoptera, excretion of uric acid, 440
respiration, 2
Hypernormal phase, starfish, 489
Hypertonic solution, defined, 45
parthenogenetic agent, 557
Hypnotoxin, 293

Hypolimnion, 226
Hyponeural nervous system, Echinoderm, 487
Hyponeural phase, starfish, 489
Hypotonic solution, defined, 45
Hypoxanthin, excreted by Mollusca, 437

I

Implantation, 536
Inadequate stimuli, starfish, 488
Infusoria, food taking, 314
Inhibitions, central, 477
  Forbes' suggested mechanism, 478, 479
  not due to stimulation of efferent fibers, 477
  peripheral, 477
  reciprocal, of antagonistic muscles, 477, 478
Inhibitor nerves—see cardio-regulator nerves.
Inogen molecule, role in muscle action, 406
Insecta, bloods, 116
  muscle types, 391
  respiration, 242
Insemination, 536
Integrations of animal bodies, 459, 460
Interactions of cells, 75
Intermediate septum, 393
Intermuscular nerve plexus, earthworm, 497–499
Internal factors of development, 544
Internal media, classification, 107
  Coelenterata, 110
  Echinodermata, 110
  mechanism for secretion, 176
  need, 106
  Nemathelminthes, 111
  Nemerteans, 111
  Oligochaetae, 112
  Platyhelminthes, 111
  Polychaetae, 112
  sponges, 109
  structure, 109
Internuncial neurones, 458
  earthworm, 502
Interoceptors, 462
Intestinal worms, respiration, 232
Intestine, excretory organ among Crustacea, 439
Intracellular digestion, 321
  Coelenterata, 323
  parasitic worms, 327
Intracellular excretion, 419
  Cephalopods, Lamellibranchs and Prosobranchs, 437
  worms, 433, 434
Intramolecular oxygen, 406
Intravasal excretophores, 430
Ionization constant, 48
Ion-protein compounds, 65, 66
Ions, 15
  calcium, in blood, 156
  concentration in blood, 152
  effects upon permeability, 49
  important in blood, 156
  potassium, in blood, 157
  present in sea water, 152
  sodium, in blood, 157
Iris, direct stimulation, 446
Iron, component of red blood, 132
Iron, significance, in haemoglobin, 127
Irreversibility, nerve trunk and reflex arc, 475
Irritability, 64
  and automatism, 380
  ciliated cells, 380
  colloidal instability, 492
  effect of fatigue, 403
  Lillie's ideas, 66
  Loeb's concept, 65
  nature, 65
  red and white muscle, 410
Isles of Langerhans, 371
Isoelectric point, 26
  proteins, 33
Isoleucine, 275
Isopods, blood, 115
  coagulation, 166
  respiration, 241
Isotonic solution, defined, 45
Isotopes, 7
  table, 7
Isotropic band, 393
Isotropic substance, muscle, 392

J

Janney, 272
Jennings, feeding Amoeba, 312
Jijima, nervous system of Planarian worm, 491
Johnson, giant fibers, 505
Jones, Lewis, dissociation, 15
Jonescu, 533
Julus, homostrophic reflex, 517
Just, 551, 554
  aster-forming substance, 552
  hypertonic sea water in artificial parthenogenesis, 556

K

Kastle and Loevenhart, 276, 338
Keber's organ, 436
Keller, 321
Kellicott, effect of temperature upon development, 565, 566
Kenyon, 518
Kidney, stones in Mollusca, 436
  theories of action, 442
  tubules, functions of parts, 442
Kite, 53, 54
Knauthe, relation of Euglena to oxygen content of water, 229
Knowlton and Moore, effect of strychnine on synaptic resistance in earthworm, 514
Koch, heart of Anodonta, 186
Kofoid, 389, 451
Kollmann, 117
Kowalewsky, chlorogogue tissue, 431
  excretion by ovoid glands, 420
  excretory organs of Crustacea, 439
Kraft, 380
Krause's membrane, 393
Krehl, 336
Krogh, 188, 190
  diffusion of oxygen, 259

Krogh and Leitch, dissociation of blood, 254
Krukenberg, 126, 137, 322, 323
  peptic ferment, 309
  uric acid in intestinal epithelium, 440
Kühne, enzymes, 264, 310
  glycogen in Myxomycetes, 350
Kukenthal, chlorogogue tissue, 431

L

Lack of oxygen, effect upon Paramoecium, 218
Lactacidogen, 407
Lactic acid, cause of fatigue, 401, 404
  content of muscle, 404, 405
  role in heart action, 200
  role in muscle action, 407, 408
  role in staircase contraction, 400
Lactic acid precursor, 407
  heart muscle, 192
Lamellibranchs, 332
  blood corpuscles, 115
  cardio-regulator nerves, 210*ff*
Lampsilis, cilia, 378–380
Langdon, 506
Langley, 456
Lankester, 126
  comment on red muscle, 410
  haemoglobin of Crustacea, 134
Latent period, 397
  effect of temperature, 398
  fertilization, 546
  red and white muscle, 410
Latham, chemical composition albumin, 60
  types of muscle fibers, 411
Lavoisier, respiration, 219
Law, conservation of energy, 11
  conservation of matter, 11
  continuity, 11
  definite proportions, 11
  gas, 12
  heart, 216
  mass action, 12
  multiple proportions, 11
  physical, in physiology, 11
  Schütz, 283
  specific nerve energies, 463
  thermodynamics, 2
Lebedinzeff, 230
Lecithin, and albumen emulsion, 58
  formula, 31
Lee, respiration, grasshopper, 247
Leeuwenhoek, 184
Leibnitz, principle of continuity, 8
Lemoine, 438
Lernanthropus, haemoglobin, 134
Lesser, respiration, earthworm, 232
Leucine, composition, 275
Leuciscus, respiration, 253
Leucocytes, 121
  instability, 122
  mast cells, 122
  polymorphonuclear, 122
  vertebrates, classification, 121
Leucocytes, transitional forms, 121
Lewes, 322
Lewis, contraction of embryonic heart, 290
Lewis, Margaret, giant cells and fibers, 433
Lieberkühn, nutrition of sponges, 321
Liebig, 219
Life, conditioned by carbon dioxide, 87
  conditioned by colloid state, 87
  conditioned by heat, 86
  conditioned by light, 86
  conditioned by oxygen, 87
  conditioned by water, 87
  conditions essential, 85
  definitions, 91, 92
  factors conditioning, 86
  general phenomena, 85
  mechanistic and vitalistic conceptions, 92
  origin a continuous process, 89
Light, effect upon development, 544
  factor conditioning life, 86
Ligia, phagocytosis, 173
  respiration, 241
Lillie, F. R., 546, 554
  fertilization, 552
  fertilizin, 552
Lillie and Knowlton, effect of low temperature upon development, 566
Lillie, R. S., 28, 474, 475, 554
  role of sodium salts on ciliary action, 382, 383
Limneus, 135
Limulus, blood, 137
  coagulation, 165
  functions of nervous system, 529, 530
  haemocyanin, 138
  heart, 205
  heart rate controlled by nerve cells, 195
  nervous system, 205, 528, 529, 530
  neurogenic heart action, 206
  respiration related to oxygen tension of water, 238
  temperature coefficient of heart action, 206
Lingle, 197
  effect of salt solutions upon turtle ventricle, 199
Linstedt, 250
Lipase, reversible action, 276
  significance of reversible action, 276
Lipoid solvents, parthenogenetic agents, 557
Lipoids, formula, 31
Lister, 310
Liver, absorption by, Daphnia, 331
  freezing point, 47
  functions, Daphnia, 331
Living matter, chemical composition, 58
  conditions demanded, 85
  fundamental properties, 64
  origin, 88
  relations of electrons and elements, 85
Llama, blood corpuscles, 117
Lobster, muscle structure, 393, 394
Local excitatory process, 464, 468, 469, 471
Locke, 197
Locomotion, role of various ganglia, crayfish, 526, 527
Loeb, J., 28, 36, 197, 554
  effect of H′ ion upon fertilization, 547, 548
  experiments on Limulus nervous system, 529, 530

Loeb, J., fertilization associated with increased oxidation of egg, 551
fertilization process, 551, 552
hydrogen ion effect on regeneration, 260
irritability, 65
method for artificial parthenogenesis, 555
oxidations of egg increased at fertilization, 553
permeability, 49
respiratory function in liver, 530
Thysanozoön, functions of brain, 511
Loeb, Leo, coagulation of blood of Limulus, 166
Loevenhart, 277
Lohman, 317
Loligo, cardio-regulator nerves, 211, 214
respiration and oxygen tension, 238
Lomonosoff, atomic theory, 6
Lotka, 301
Lowe, 274
Lucas, 468, 474, 475
all-or-none law, striated muscle, 403
gas chamber experiment, 469
local excitatory process, 464
Luciani, 244, 245
Ludwig, ideas on excretion, 443
Lumbricidae, digestive system, 330
Lumbricus, cardio-regulator nerves, 208
centralization of nerve elements, 492
cephalization, 492
cross section of nerve cord, 500
minute structure of nerve cord, 501
nephridium, 427, 428
oxygen tension, 250
sense bud, 494
sensory processes and nerve fibers, 494
striated borders of epithelium cells, 330
structure of ganglia, 506
Lunar period, effects on spawning reactions, 541, 542
Lunar periodicity of human births, 543
Lund, oxygen consumption of Paramoecium, 234
Lung, freezing point, 47
Lung-fish, vascular system, 182, 183
Lungs, 222
Lutz, threshold value in reflex, 476
Lymph, 108
freezing point, 47
Lymphocytes, phagocytic powers among worms, 433
vertebrate, 121
Lysin, role in fertilization, 551
Lysine, composition, 276

M

Macallum, 150, 151
McBride, hormone substances in development, 575
McClendon, 68, 274, 554,
sea water a balanced solution, 569
MacDonald, 389
Machines, living and lifeless, 296
Macrogamete, 536
Macrosomes, 55
Mactra, 556
Madsen and Watabaki, 174
Magnesium, effect upon ciliary action, 383
Maia, carbon dioxide content of blood, 164
coagulation of blood, 166
pigment of blood, 137
Malfitino, G., 26
Mall, factors responsible for production of monsters, 566
Malpighi, 184
Malpighian corpuscle, 435
Malpighian tubes, 440
structure, 441
Mammal, chemical composition, 58, 59
circulating fluid, 100
circulatory system, 98
excretory system, 101
nervous system, 103
reproductive system, 105
respiratory system, 99
Mantis, excretion, 441
Marcet, 335
Marchal, 439
excretion by intestinal epithelium, 440
Marchesini, 123
Marginal nerve ring, Gonionemus, 483
Marine protozoa, no excretory organ, 413
Mass action, law, 12
Mathews, 554
adsorption, 22, 23
secretion, 290, 291
Matter, states of, and their combinations, 18
structure, 5
Matula, experiments upon Sicyonia, 527
Maturation, 536, 538, 539
Maxwell, work done by cilia, 581
Mayer, rhythmical pulsations of medusae, 485
Mayo, John, respiration, 219
Meade, 554
Mechanical agitation, parthenogenetic agent, 557
Mechanism, 450
Mechanists, 449
Medulated sheath, function, 467
Medusa, nervous system, 483, 484
Meeh's formula, 357
Membranelles, 377
Membranes, collodion, 28
defined, 40
elevation in fertilization, 547
semipermeable, 40, 41
significance in irritability, 66
Memory, 70
Merunowicz, 197
Mesenterial filaments, Actinians and medusae, 325
digestion, 322
Metabolism, 63, 68, 85, 219
and energy, 220
associated with spawning, 542
carbohydrate, 349
effect of external temperature, 356
general conclusions, 220
general results, 349
regulated by thyroxin, 366
Metalnikow, 308
digestion in Paramoecium, 315
Metazoa, reproduction a series of processes, 536

Metchnikoff, 321, 323, 327
Methaemoglobin, 127
Metridium, rate of transmission of nerve impulse, 486
  reversal of ciliary action, 387
Meyer, 274
Miall, 243
Microdissection, 53, 54, 55
Microgamete, 536
Microsomes, 55
Mid gut, function, worms, 330
Middle piece, 540
Migration of nerve cell bodies, 481, 482
Millikan, 9, 60
Millon's reaction, 34
Milne-Edwards, 112, 126
Mines, 197
  balance of ions in saline solutions, 199
  excitation process and contractile process, 398
Mines' solution, effect on heart of frog, Fig. 28, 192, 193
Minot, 71
Mitchell, 346
Molecular weight, colloids, 28
  proteins, 28
Molecules, structure, 7
Mollusca, alimentary system, 96
  blood corpuscles, 115
  circulating fluid, 100
  circulatory system, 97
  excretion, 435*ff*
  excretory system, 101
  nervous system, 102
  phagocytosis, 173
  reproductive system, 104
  respiratory system, 99
  vascular action, 186
Molschott, 245
Monaco, 245
Monk, 336
Monsters, produced by temperature changes, 555, 556
Moore, A. R., 456, 457, 554
  carbon dioxide production by nerve, 465
  experiments upon Yungia, 511
  homostrophic reflex, worms, 517
  muscle tension in worms, 516
  reciprocal inhibition in Antedon, 491
  righting reactions of starfish, 489
Moore, Benjamin, 9, 88, 302, 450
  experiments upon synthesis, 90
  synthesis by colloids, 535
Moore, Edie, Whiteley and Dakin, review of Pütter's work, 318
Moore and Rockwood, 336
Morawitz, 170
Morgan, 554, 576
Morgulis, homostrophic reflex, worms, 517
Motor neurones, position of cell body, 481, 482
Moulting, effect on respiration, 245
Mouton, tryptic enzyme in Amoeba, 313
Movement, adaptation, 77
  physiology, 373
Mucin, 293
Mucous, 292
Mucous secretions, 294
Müller, 322, 361
  specific nerve energies, 463
Multiple proportions, law, 12
Multiple synapse, 505
Multipolar cells, 507
Murexide, 293
Muscle, action, 395*ff*
  all-or-none law, 402, 403
  anistropic substance, 392
  apparent reversal of bands, 394
  behavior of different types, 389
  cardiac, 388
  cells, 79
  clonus, 401
  contracture, 404
  curve of extension, 395
  direct stimulation among sponges, 446
  distribution of different types in animal kingdom, 389
  effect of osmotic pressure on cardiac muscle action, 386
  effect of temperature upon tetanus, 402
  elasticity, 395
  excitation and contraction processes, 398
  extensibility, 395
  fatigue, 401, 403
  fibrillae, 391
  forms of contraction, 396
  freezing point, 47
  fundamental physiological properties 388
  heat produced by isotonic and isometric, contractions, 408
  heat produced during recovery, 407
  heat production, 215
  heat production proportional to length of fiber, 408
  isometric contraction, 396
  isotonic contraction, 396
  isotropic substance, 392
  latent period, 397
  lobster, 393, 394
  nuclei, 391
  osmotic pressure, 404
  oxidation processes, 408
  rate of stimulation by nerve impulses, 402
  rate of stimulation inducing tetanus in various forms, 402
  red and white, 410
  refractory phase, 401
  smooth, 388
  staircase contraction, 400
  striated or voluntary, 388
  striated, structure, 391*ff*
  structure, 391*ff*
  summary of phenomena of twitch, 409
  summation of contractions, 400
  superposition of contractions, 400
  temperature coefficient, 398
  tonicity, 398, 399
  twitch, 396, 397
  types, 388
Muscle action, 395*ff*
  role of lactic acid, 407
Muscle cells, Hydra, 389

Muscle nuclei, 391
Muscle tension, 407
Muscle tension and reflexes in worms, 516
Muscle twitch, red and white muscle, 410
Muscular fatigue, 403
Musculo-epithelium, 78
  hydroids, 390
Mushroom bodies, bee, 533
Mustelus, carbon dioxide production of nerve, 465, 466
Mya, heart nerves, 210, 211
  respiration, 240
Myers, chemical composition of blood, 144
Myogenic theory of heart beat, 197
Myriopoda, blood, 116
Mysis, antennal gland, 438
Mytilus, blood, 115
  effects of ions on cilia, 382
  effect of temperature on ciliary action, 384, 385
  energy for ciliary action of protein origin, 385
  inhibitory nerves, 210, 211
  irritability of ciliated cells, 380
Myxomycetes, 78
  peptic digestion, 309
  reactions of food vacuoles, 310
  starch digestion, 316

N

Nawrocki, 132
Nelson, 332
Nemathelminthes, alimentary system, 96
  circulating fluid, 100
  circulatory system, 17
  excretory system, 100
  internal media, 111
  nervous system, 102
  reproductive system, 104
  respiratory system, 98
Nemerteans, internal media, 111
Nephridia, 423
  correlation between tube length and life habit, 434
  disposal of solid particles, 433
  function in water conservation, 434
  functions of flame cells, 428
  functions of parts, 428
  functions of solenocytes, 428
  functions of terminal cells, 428
  Lumbricus, 427, 428
Nephrocytes, 117
  Echinoderm, 420
  specific action, 420
Nephrostome, 425, 426
Nereis, 556
  cardio-regulators, 208
  centralization and cephalization, 493
  cross section of egg, showing sperm, Fig. 144, 545
  fertilization, 546
  respiration, 237
  Rouget cells, 190
  spawning, 542
Nerve action, continuous decrement, 469
  electrical changes involved, 464
Nerve action, local excitatory process, 464
  nature, 467
  oxygen necessary, 464
  propagated disturbance, 464
Nerve, carbon dioxide produced by injury, 465, 466
Nerve cells, bipolar, 507
  Corydalis, 524
  effects of nutrition, 466, 467
  multipolar, 507
  rate of discharge, 468
  types in earthworm ganglion, 507
  unipolar, 507
Nerve cord (earthworm) described, 502
  minute structure, 501
Nerve fibers, distribution of tracts in Branchipus, 521
Nerve impulse, rate of conduction, 464, 467, 472, 478
  discussion, 468–472
Nerve net, 452, 453
  applicability of term to giant fiber system, 506
  Coelenterata, 482
  distribution in animal kingdom, 453
  distinguishing features, 455
  Echinodermata, 486
  existence among vertebrates, 455
  found in capillary wall, 178
  function in blood capillaries, 178
  transition to synaptic system, 456
Nerve net and nerve plexus, 497
Nerve plexus systems, earthworm, 497, 498
  factor in coordination, 512
  guinea pig, 497
  subepithelial, earthworm, 498
Nervous conduction, chain of elements involved in earthworm, 508
  trunk and reflex arc compared, 473, 474
Nervous organization, 454
  types, 480
Nervous system, 446*ff*
  Amphibia, 103
  Annelida, 102
  Aves, 103
  centers of control in Limulus, 528
  chemical differentiation, 456, 457
  Coelenterata, 102
  Corydalis, 521–525
  crayfish, 525
  Crustacea, 102
  Cyclostoma, 103
  earthworm, anterior end, 499
  earthworm, operation, 510–517
  Echinodermata, 102, 486
  Elasmobranchii, 103
  experiments upon crayfish, 526
  functions in earthworm and Nereis, 513
  Hemichordata, 102
  Hexapoda, 102
  honey bee, 531–533
  ladder type, 518*ff*
  leech unlike Oligochaetae, 494
  Limulus, 528, 529, 530
  Mammalia, 103
  Medusa, Gonionemus, 483, 484

Nervous system, Mollusca, 102
need for, 446, 447
Nemathelminthes, 102
Pisces, 103
Platyhelminthes, 102
Porifera, 101
Protozoa, 101
receptor-adjustor-effector system, 460
reptilia, 103
structure of ganglion of earthworm, 506
Urochordata, 102
worms, 491*ff*
Nervous tissue, fundamental properties, 446
Nervous transmission, rate in Metridium, 486
Neuport, 243
Neurite, 458
Neuroid conduction, cilia, 380
Neuromotor apparatus, 377, 389, 451, 452
Neurone system, merits, 458
Neurone theory, 457
Neurones, 453
adjustor, 458
afferent, 458
ascending and descending, earthworm, 514
classification, 458
diagrammatic representation, 457
efferent, 458
functions, 502
internuncial, 458
Purkinje cell, 459
pyramidal, 459
Neuropile, 502
functions, 510
Neutral red, use in studying digestion in protozoa, 269
Newport, respiration of larvae, 233
Nierenstein, 315
Nissl granules, role in nerve action, 467
Nitrates, 302
Nitrites, 302
atmospheric, used by plants, 301
Nitrobacter, 302
Nitrogen, atmospheric, not used by animals, 219
Nitrogen cycle, 301
Nitrogen equilibrium, 347
Nitrogen fixers, 302
Nitrosomonas, 302
Nonelectrolytes, cardiac muscle, 386
ciliary action 386
Nuclear membrane, 73
Nuclear spindle, 82
Nucleases, 267
Nuclei, muscle, 391
Nucleus, function, 73
gel structure, 72
role in oxidation, 74
structure, 54, 55
synthesizing organ, 73
Nussbaum, 290
Nutrition, 296*ff*
complexity of food stuffs needed, 296
different animal groups, 308
dissolved organic matter, 317
elements demanded in food, 296
protozoa, 308
Nutrition, purpose, 296
sponges, 316

O

Octopus, carbon dioxide content of blood, 164
haemocyanin, 138, 137
haemocyanin crystals, 138
Odonata, respiratory system, 250
Oils, 293
Oligochaetae, excretophores, 431
internal media, 112
Oniscus, respiration, 241
Oogonia, 538
Ophelia, 556
Opisthobranchs, excretory organs, 435
Opsonin, 173
Oral branchiae, as excretory organs, 418
Organ formation, 536
Organ forming substances, 567
Organ systems, 94*ff*
survey, 96*ff*
Organic vs. inorganic, 8
Organism, a mechanism, 450
conceived as a machine, plus, 449
Organization, significance of polarity, 492
Organs and tissues, 94
Origin of heart beat, 196
Orthoptera, 246
Osmodesma, 440
Osmosis, 40
role in physiology, 50
Osmotic pressure, 41
body fluids and sea water, 143, 146–150
colloids, 26
defined, 43
determination, 44
effect upon cardiac muscle, 386
effect upon ciliary action, 386
effect upon development, 544, 561
relation to freezing point, 44
resting and fatigued muscle, 404
table, 45
tissues, 47
Van't Hoff's theory, 41
Ostrea, blood, 115
Ovoid gland, excretory organ in Echinodermata, 420
Ovulation, 536
Oxidations, physiological, 359
Oxygen, absorption by water, 224
amount used in oxidizing foods, 220
condition of life, 87
consumption and concentration, 237
consumption by animals at different temperatures, 257
consumption by fish, 251
consumption during development, 258
consumption in different animal groups, 236
content of lake waters, 225*ff*
content of various bloods, 141
content of water, variations, 228
diffusion into water, 229
effect upon cardiac muscle, 386
effect upon ciliary action, 385, 386

Oxygen, effect upon development, 544, 570
effect of high concentration upon protozoa, 235
effect of lack, upon protozoa, 218
in depths of ocean, 238
intramolecular, 406
need, 218
not the cause of oxidations, 219
solubility in water, 124, 125
Oxygen capacity, Arenicola haemoglobin, 132
Cheironomous blood, 134
Planorbis, 134
Oxygen cycle, 301
Oxygen tension, blood of sharks, 252
tissues of invertebrates, 250
Oxyhaemoglobin, 127
dissociation, 128
Oxyproline, composition, 276

P

Pacemaker of heart, invertebrates, 206, 207
Palaemon, coagulation of blood, 166
diagram of circulation, 179
Palaemonetes, respiration and oxygen tension of water, 238
Palinurus, carbon dioxide content of blood, 164
cardio-regulators, 212
coagulation of blood, 166
Palolo worm, spawning, 542
Paludina, 135
Pancreas, 371
Paphia, 240
Paraglycogen, Gregarines, 351
Parajulus, homostrophic reflex, 517
Paramoecium, 70
choice of food, 316
circulation of endoplasm, 315
contractile vacuole, 413
digestion, 314
excretion crystals, 415
neuromotor apparatus, 452
rate of operation of contractile vacuole, 414
rhythms in reproduction, 69
Paramylum, 350
Parasitic worms, intracellular digestion, 327
Parasitism, effects upon digestion, 329
Parathyroid glands, 370
results of removal, 371
role in calcium metabolism, 371
Parhon, M., respiration of bees, 250
Parker, 316, 317, 452, 453, 456
carbon dioxide production by nerve, 465, 466
circulation in sponges, 177
complexity of organization and behavior, 451
coordination of cilia, 380
position of nucleated portion of neurone, 481
rate of nervous transmission in Metridium, 486
receptor-effector mechanisms, 480, 481
reversal of ciliary activity, 387
Rouget cells, 190
Parsons and Parsons, 164
Parthenogenesis, natural, 557
Parthenogenetic agents, 555
Partial pressure, gas, 223
Pasteur, 263
Pasteur, biogenesis, 534
Pasteur and Roulin, uric acid in silk worm, 440
Patten, experiments upon the nervous system of Limulus, 430, 529
Patten and Redenbaugh, 528
Pavlov, 289
digestion, 270
Pearl, 292
experiments upon Limulus, 529
Pecten, 332
Pedicellariae, reflex stimulation, 488
Pelomyxa, 314
diastatic ferment, 310
Penetration of spermatozoon, 546
Pentosans, 31
Pepsin, effect upon proteins, 272
Peptic digestion, invertebrates, 271
Perch, respiration, 251, 253
Perfertorium, 540
Pericardial fluid, 293
Pericardial gland, Mollusca, 435, 437
Peripheral nerves, earthworm, 500
Peripheral resistance, 191
how varied, 191
Peristalsis, Daphnia, 331
Perivitelline fluid, 545
Permeability, ciliated cells by H′, OH′ ions, 383, 384
direct determination, 50
factors influencing, 49
and irritability, 67
membrane, 48
Perspiration, 293
Peters, 308, 406
nutrition of Protozoa, 297
Petromyzon, 265, 556
Pettenkofer, 219, 244
and Voit, 220
Pfeffer, 41
Pflüger, 231
ideas concerning muscle action, 406
intramolecular oxygen, 406
Phagocytes, amoeboid cells of coelomic fluid, 432
carriers of excreta, 430
Echinodermata, 419
higher animals, 174
intracellular digestion, 270
nephridial cells, 429
Phagocytic action of tissue cells, 173
Phagocytosis, 172
carbon and quartz, 174
chance of collision, 174
chemical in character, 172
effect of temperature, 174
role of surface tension, 175
Phallusia, blood, 117
Phase, continuous, 14
dispersed, 14
relations in cell division, 83
reversal, 56, 57
Phenylalanine, composition, 275
Phoronis, haemoglobin, 131
Phosphates, as buffers, 161, 162
needed for growth, 298

Phosphoric acid, excreted by Mollusca, 436
part in muscular fatigue, 401
Phosphorous cycle, 303
Photoreceptors, earthworm, 495, 496
Photosynthesis and oxygen content of water, 229
Physical change involved in fertilization, 546
Physiological oxidations, 359
Physiological properties, muscle, 388
protoplasm, 63*ff*
Physiological saline solution, effect on crab heart, 198
effect on turtle ventricle, 199
Physiologically balanced solutions, 153, 154
Physiology, classification, 2
comparative, aims, 4
defined, 1
point of view, 1
relations to other biological sciences, 3
relations to physical sciences, 4
scope, 3
Pigment cell, compared to smooth muscle, 389
Pilocarpine, effect upon sweat glands, 291
Pine, chemical composition, 58, 59
Pineal gland, 371
inhibitory action, 371, 372
Pinnaglobin, chemical composition, 126
Piper, rate at which nerve impulses enter muscle, 402
rate of motor impulses, 468
Pisces, alimentary system, 96
circulating fluid, 100
circulatory system, 98
excretory system, 101
nervous system, 103
reproductive system, 105
respiratory system, 99
Pituitary gland, 369
hormones, 369
influence upon physical features, 370
Pituitrin, 369
Placentation, 536
Planaria, digestion, 327
nervous system, 491
respiration, 239
types of cells in alimentary tract, 327
Plankton, as food, 317
Planorbis, 135
haemoglobin, 133
Plant, 338
Plateau, 440
Platyhelminthes, alimentary system, 96
circulating fluid, 100
circulatory system, 97
excretory system, 100
internal media, 111
nervous system, 102
reproductive system, 104
respiratory system, 98
Pneumatophores, excretory structures, 417
Poikilothermous animals, 352, 353
Poisons, 293
Polarity, differential metabolic activity, 492
nerve cells, 456
nerve net, 456
significance in nervous organization, 492
Poll and Sommer, 369
Polychaetae, centralization, 492
cephalization, 492, 493
excretophores, 431
Polydesmus, homostrophic reflex, 517
Polyneuritis, birds and mammals, 306
Polynoe, 556
Polyphemus moth, cardio-regulator nerves, 213
Pomeroy, Rouget cells in Nereis, 190
Ponder, 135
Porania, pH of pyloric coeca,
Porcellio, respiration, 241
Porifera, alimentary system, 96
circulating fluid, 100
circulatory system, 97
excretory system, 100
muscle, 389
nervous system, 101
reproductive system, 104
respiratory system, 98
Porpita, excretion, 416
Porpites, 325
Portunus, 137
gills, 242
Potassium, diffusion, 50
effect upon heart muscle, 199
effect upon respiration, 240
effects upon development, 572
Powers, respiration of fish, 253
Pratt, excretion, Alcyonium, 416
Predigestion, 325
Pressure, effect upon development, 561
role in excretion, 421
Preyer, 132
Priestley, respiration, 219
Proferments, 267
Progressive movements, 513
Proline, composition, 276
Promorphology of egg, 574
Propagated disturbance, 464, 468, 471
associated with refractory period, 470
rate of conduction, 472
Proprioceptors, 463
Prosobranchs, cardio-regulator nerves, 210, 212
Protein-ion compounds, 26
Proteins, 31, 32
adequate and inadequate, 343
amphoteric electrolytes, 33
biological value, 346
chemical properties, 33
classification, 34
coagulation temperature, 32
colloidal behavior, 34, 35
color tests, 33, 34
crystallizable, 32
factors determining food value, 342
molecular weight, 28
not built in body, 343
percentage composition, 344, 345
physical properties, 32
salting out, 32
structure, 343
synthesis and specificity, 275
Prothrombin, 171
Protoneurone, 452, 458

Protoplasm, 52
alveolar theory, 53
catalytic agents, 64
chemical composition, 58, 63
cyclic changes, 63
heterogeneous system, 61
hydrophyllic emulsion, 55
metabolism, 63
properties, 52, 63
responsive to stimulation, 447
reticular theory, 52
structure, 52
Protoplasmic continuity, nerve net system, 458, 459
Protoplasmic movements, 374
Protozoa, alimentary system, 96
circulating fluid, 100
circulatory system, 97
excretion, 413
excretion by crystal or granule formation, 415
excretory system, 100
feeding experiments, 309
nervous system, 101
neuromuscular apparatus, 451
nutrition, 308
respiratory system, 98
reproductive system, 103
Pseudopod, 78
Pulmonates, 435
Punktsubstanz, Corydalis, 524, 525
Purkinje, 52
Purple dye, 293
Pütter, nutrition of aquatic forms, 317

Q

Quatrefages, 126

R

Rachford, 336
Radial nerve tissue, Gonionemus, 484
Rana, 556
Ranvier, duration of twitch in red and white muscle, 410
Rare earth metals, heart action, 202
Rate of conduction, reflex arc and nervetrunk, 474
Rate of transmission, 55
effect of synapse, 515
Helmholtz, 464
Metridium, 486
variability due to structure, 515
worms, 516
Receptors, bee, 533
cephalization, 492
circulatory sensations, 463
classes and limitations, 461, 462
contact, 462
earthworm, 495*ff*
Echinoderm, 487
graded sensitivity, 492
hunger, 462
light, 462
nausea, 463
Receptors, position, 462
proprioceptors, 463
respiratory sensations, 463
sexual sensations, 463
sound, 462
temperature, 462
thirst, 463
types in Limulus, 528
visceral pain, 463
Reciprocal inhibition, starfish, 488
Reciprocal innervation, 514
Red corpuscle, evolution, 119
number, 118
shape, 118
size, 118
Reduced haemoglobin, 127
Rees, neuromotor apparatus of Paramoecium, 452
Reflex, 472
and muscle tension in worms, 516
crossed extension, 478
homostrophic, worms, 517
not always predictable, starfish, 488
Reflex action, starfish, 488
Reflex arc, contrasted with peripheral nerve, 474
rate of transmission compared with nerve trunk, 473, 474
Reflex inhibition, 477
Reflex mechanism, hydroid, 482
variations in arrangement, 473
Refractory period, 468, 470
absolute, 470
relative, 470
temperature coefficient, 470, 471
time relations, 470
Refractory phase, muscle, 401
role in convergence of impulses, 476
role in nerve net, 456
starfish, 488
Regeneration, effect of H′ ion, 260
Region of decrement, earthworm, 515
Regnard, 134
Regnault, 219
and Riset, 243
Rehburg, endothelium of capillary, 189
Reinforcement and inhibition, nerve trunk and reflex arc, 477
Rejuvenation, 536
Rennase, 286
Rennin, identity with pepsin, 285
Reproduction, 71
fundamental types, 535
physiology, 534
type of behavior, 543
Reproductive system, Amphibia, 105
Annelida, 104
Aves, 105
Coelenterata, 104
Crustacea, 104
Cyclostoma, 105
Echinoderm, 104
Elasmobranchii, 105
Hemichrodata, 104
Hexopoda, 104
Mammalia, 105
Mollusca, 104

Reproductive system, Nemathelminthes, 104
Pisces, 105
Platyhelminthes, 104
Porifera, 104
Protozoa, 103
Reptilia, 105
Urochordata, 104
Reptilia, alimentary system, 97
circulating fluid, 100
circulatory system, 98
excretory system, 101
nervous system, 103
reproductive system, 105
respiratory system, 99
Resistance to blood flow, 191
Resorption, nephridial tube, 429
Respiration, aerobic, 231
anaerobic, 231
aquatic animals, 251
aquatic forms and oxygen tension of water, 238
Ascaris, 232
Asellus, 241
calorimeter, 348
crabs, 242
crustacean, 241
defined, 218
diffusion of oxygen through respiratory epithelium, 259
diving beetles, 245
Dytiscus, 245
earthworm, 239
effects of H′ ion upon fishes, 253
effects of season, on bees, 250
effects of temperature, on bees, 250
external, 218
fish, 251
frogs in nitrogen, 231
grasshopper, 246, 247, 248
hibernating animals, 256
historical statement, 219
insects, 242
internal, 218
intestinal worms, 232
Isopods, 241
Leuciscus, 253
Ligia, 241
lower animals at definite temperatures, 257
lower land animals, 243
necessary, 218
Oniscus, 241
Paramoecium, 234
pike, 251
Planaria, 239
Porcellio, 241
related to rate of diffusion, 238
role of H′ ion, 260
sea urchin eggs, 238
seasonal variation in silk worm, 244
silk worm, 243, 244
tench, 251
tracheal system, 242
trout, 252
vinegar eel, 232
Respiratory mechanism, control in Limulus, 530
Respiratory organs, types, 221, 222
Respiratory papillae, 221
as excretory organs, 418
Respiratory proteins, 125
Respiratory quotient, 220
respiration of bee, 250
variations due to food consumed, 220
Respiratory surface, crabs, 241, 242
Respiratory system, Amphibia, 99
Annelida, 99
Aves, 99
Coelenterata, 98
Crustacea, 99
Cyclostoma, 99
Echinoderm, 99
Elasmobranchii, 99
Hemichordata, 99
Hexopoda, 99
Mammalia, 99
Mollusca, 99
Nemathelminthes, 98
Pisces, 99
Platyhelminthes, 98
Porifera, 98
Protozoa, 98
Reptilia, 99
Urochordata, 99
Respiratory tree, 222
as excretory organ, 418
Rete mirabile, swim bladder, 255
Reticulitermes. 298
Retzius, 506, 518
Rouget cells, 190
Reversal of phase, 57
Rhizopods, food, 309
Rhythmical pulsation, 485
role of sodium ions, 485
stimulation provided through nerve net, 485
Rhythmicity, 69
Rickets, 306
Riddle, rate of digestion in cold blooded animals, 332
Ringer, 197
Riset, 219
Roaf, 332
enzymes of invertebrates, 281, 282
method of study of enzyme actions, 283
Rod canal, functions, 428
nephridium of earthworm, 428
Rogers, 197
Rollet, 132
Rosa, 219
Rouget, 112
cells, 188, 189
Rouget cells, sympathetic innervation, 190
Rubenstein, automatic movement and irritability, 380
Rubner, 219, 349, 358

S

Sach's rule, 559
Saguchi, cilium structure, 377, 378
Salamander, rate of digestion, 332
vascular apparatus, 183
Salt content of blood and of sea water, 148

Salt content of body, regulation, 412
Salts, as food, 305
  effects on ciliary action, 382
  effects of heart action, 197
Sanford, 335
Sarcolemma, 391
Sarcomere, 392
Sarcoplasm, 391
Sarcostyles, 391
Sarcous element, 392
Sartorius, heat production, 410
  temperature coefficient, 398
Saxidomus, 240
Schaeffer, amoeboid movement, 375
Schneider, nerve net structure, 454
Schultz, phagocytosis in Echinoderms, 419
Science, exact, 2
  observational, 2
Schleiden and Schwann, 72
Schultz' law, 283
Scolopendra, excretion, 441
Scott, causal agents of fatigue, 404
Scott, G. G., 142, 250
Scott and Kellicott, oxygen consumption by Fundulus, 258
Sea urchin, excretion, 420
Sea water, McClendon's, 154
Season, effects on respiration of bees, 250
Secretin, gastric, 292
Secretion, 287*ff*
  appearance of cells, 295
  changes of permeability during discharge, 294
  chemical stimulation, 292
  classification, 292, 293
  cytolytic effect, 293
  defined, 287
  how induced, 289
  kinds, 292
  mucous, 294
  nerve stimulation, 289
  period of discharge, 293
  period of recovery, 293
  phases, 289
  poisons, 293
  psychical or appetite, 289
  role of muscle fibers, 290, 291
  salivary glands, 290
  serous, 294
  sign of irritability, 288
  special, 293
  unicellular glands, 290
Secretogogues, 292
Segmental ganglia, bee, functions, 533
Selective diffusion, excretion, 422
Semimembranosus, heat production, 410
Seneca Lake, oxygen content, 227
Sense organs, importance, Gonionemus, 484
Sensori-motor system, earthworm, 497
Sensory neurones, position of cell body, 481, 482
Sepia, haemocyanin, 138
Serine, composition, 275
Serous secretions, 294
Serpula, blood pigments, 127
Sexual reproduction, 536
Sharp, 389, 451
Shell glands, 438
Sherrington, 474, 477, 514
  fatigue in reflex action, 475
Sicyonia, experiments upon nervous system, 527
Silk, 292
Silkworm, respiration, 243, 244
Simpson, 355
Sino-auricular junction, effect of ligature, 206
Sino-auricular node, 207
Sinus, beat, 206
  functions, 290
Siphonoglyphes, cilia, 387
Siphostoma, 126
Sipunculus, oxygen consumption, 237
Skin, as excretory organ, 439
Smallwood and Rogers, 466, 467
Snodgrass, nervous system of honey bee, 531–533
  vascular apparatus of bee, 181–183
Snyder, heat production in terrapin heart, 215
Sodium: calcium ratio, effect upon Yungia, 511
Sodium, diffusion, 50
  effect upon ciliary action, 382, 383
  effect upon heart action, 197, 199
  effect upon muscle tone, 399
Sodium ions, role in rhythmical pulsations, 485
Sodium standstill, 199
Sol, defined, 14
Solenensis, 135
Solenocyte, 426
  functions, 428
Sols and gels, 18
Solute, defined, 13
Solution, defined, 13
  Fuhner's, 155
  Göthlin's, 155
  hypotonic, 45
  isotonic, 45
  Locke's, 155
  McClendon's, 154
  Mines', 156
  Ringer's, 155
  Tyrode's, 155
  Van't Hoff's, 155
Solutions, 13
  classification, 14
  colloidal, 14
  crystalloidal, 14
  electrolytic, 14
  non-electrolytic, 14
Solvent, defined, 13
Sorensen's nomenclature, 158
Sorg, 243
Spallanzani, 243
Spallita, gas exchange in turtle, 259
Spawning, effects of lunar period, 540–543
Special functions, 75
Specific nerve energies, law, 463
Spectrum, Arenicola haemoglobin, 132
  ethereal vibrations, 461
  haemocyanin, 138
  haemoglobin, 128
Sperm cell, 80
Sperm dilution experiment, 552, 553
Sperm shedding, 541
Spermatogonia, 538

Spermatozoa, effect of H′ ions upon respiration, 258
effects of H′ ion, 382, 260
entrance into egg, 545
number required for fertilization, 552, 533
structure, 540
types, 538, 540
Spherule cells, 116
Spicules, 292
Spider web, 292
Spinal centers, Forbes' scheme of organization, 479
Spindle cells, frog, 168
Spiral valve, frog, 186
Spirographis, 126
Spleen, freezing point, 47
Sponges, circulation, 177
diastases, 322
enzymes, 322
excretion, 415, 416
food taking, 320
movement of water through body, 316
no nerve structures, 446
nutrition, 316
receptor-effector mechanisms, 480, 481
Spongin, 292
Spontaneity, 68
Spontaneous generation, 88
Sporulation, 535
Squinado, coagulation of blood, 166
Staircase contraction, 400
effect of carbon dioxide, 400
prolonged in presence of oxygen, 400
Staircase effect in ciliary action, 381
Starch, digestion, 277
Starfish, reflex actions, 488
Starling, 188, 292
law of heart, 216
Status lymphaticus, 365
Sterilization, a factor in fertilization, 546
Stimulation, chemical, 511
limits in earthworm, 515
rate proportional to absolute temperature, 402
through anesthetized regions, 514, 515
Stimuli, classification, 65
Stockard, critical periods of development, 560
Storage excretion, Echinoderms, 420
Stough, giant fibers of earthworm, 503*ff*
Stout, 71
Strassburger, 52
Streaming movements, 373
associated with fertilization, 546
Chara and Nitella, 373
theories, 373
Striated borders, epithelium cells, 330
Strongylocentrotus, 556
effect of temperature upon cleavage, 564
excretion, 420
Strontium, in physiological saline solutions, 200
Strychnine, effect on nervous system, 491
role in indicating synaptic relations, 456
Style sac, 332
Stylonichia, 309
effect of temperature on contraction of contractile vacuole, 414
Stylotella, effect of stimulation upon oscula, 390
Sub-epidermal nerve pelxus, earthworm, 497
role in reflexes, 516
Suberites, carbon exchange, 317
nutrition, 317
pepsin, 322
Suboesophageal ganglia, role in Limulus, 530
Sugar, functions in animal body, 351
Sugar metabolism and pancreas, 371
Sulphates, diffusion, 50
Sulphion, effects upon development, 573
Summation, nerve trunk and reflex arc, 474
Summation of contractions, 400
Summation of propagated disturbances, 474, 475
Summation of stimuli, starfish, 488
Surface tension, 25
part played in muscle action, 407
Suspension, 14, 17
and suspensoid, 19
sedimentation, 19
size of particles, 19
Swammerdam, 131
Swezy, 389
Swimbladder, functions, 255
gases contained, 255
Sycon, trypsin, 322
Sycotypus, cardio-regulator nerves, 210, 212
Symbiosis, a factor in origin of erythrocytes, 120
termites and protozoa, 299
Sympathetic innervation of capillaries, 190
Sympathetic nerve in secretion, 290
Sympathetic nervous system, Corydalis, 523
Synapse, 458, 459
origin, 456
Synapsis, giant fibers, 505
Synaptic membrane, 459
Synaptic nervous system, 452
transition from nerve net, 456
Syncytium, 378
cardiac muscle, 388
Synovial fluid, 293
Synthesis, role of colloids, 90
role of nucleus, 73
Synthetic enzymes, 277
Systems, dispersed, 17
heterogeneous, 17, 18
homogeneous, 16

T

Table, conversion, per centage NaCl and Molecular concentration, 156
Tail, spermatozoon, 540
Tait, phagocytosis, 173
Tapes, heart nerves, 210, 211
Tashiro, carbondioxide produced by nerve, 465, 466
metabolic activity of Palolo worm, 542
Taurine, excreted by Mollusca, 436
Taylor, 389, 452,
sol-gel reversibility of contractile vacuole, 413
Tedania, 322
Teichmann's test, 132
Temperature, (absolute) influence on rate of stimulation of muscle, 402

Temperature, body in relation to environment, 194, 195
correspondence between sea water and animals, 157
effect of change, on sinus, 206
effect on cardiac muscle, 386
effect on chemical reactions, 194
effect on ciliary action, 384, 385, 386
effect on contraction of muscle, 398
effect on development, 544, 562–566
effect upon frog's Gastrocenmius, 399
effect upon heart action, 194, 196
effect upon irritability of muscle, 398
effect upon latent period, 398
effect upon muscle contraction, 398
effect upon oxygen consumption, 257
effect upon relaxation of muscle, 398
effect upon spawning, 542
limits for life, 86
maintenance, 69
variations in man, 354
Temperature coefficient, 194
effect upon cleavage of Arbacia and Strongylocentrotus, 564
formula, 195
heart action, 195
heart action of Limulus, 206
latent period, 398
movements of mouth parts in Echinus, 284
muscle contraction, 398
pulsation of contractile vacuole in protozoa, 414
Temperature maintenance, role of nervous system, 354
Temperature receptors, 462
Tench, respiration, 251
Tension, developed during stimulation, 407
due to lactic acid, 408
gas, 223
unloading, 254
Terebella, blood pigment, 127
Terminal cell, excretory, 423
nephridia, 428
Termites, effect of oxygen on intestinal fauna, 235
nitrogen-free diet, 298
nutrition, 298
Termopsis, 298
Tests, biuret, 34
Millon's, 34
thiophene, for lactic acid, 405
xanthoproteic, 34
Tetanus, development, 400
genesis, 401
partial, 401
rate of stimulation demanded, 402
smooth muscle, 402
Tethelin, 369
Thalassema, 556
Thermocline, 226
Thermodynamics, laws, 12
Thigmocytes, crayfish, 114
Thiophene, test for lactic acid, 405
Thomas, Karl, 346
Thomson, 309
Threshold, nerve trunk and reflex arc, 475
Threshold bodies, 444, 445
Threshold of stimulation, 463
Thrombin, 168
Thrombocytes, 122, 172
Thrombokinase, 171, 172
Thunberg, 237
Thymus gland, 364
effect of under-feeding, 366
functions, 365
occurrence, 365
Thyroid gland, 366
experiments of Gudernatsch, 367
functions, 366, 367
Thyroxin, 366
Thysanozoön, brain, 511
Timm, chlorogogue tissue, 431
Tissue cells as phagocytes, 173
Tissues, morphological classification, 94
and organs, 94
physiological classification, 94
Tonicity, muscle, 398
Tonus, capillary walls, 189
effect of calcium salts, 399
effect of sodium chloride, 399
Tortoise, rate of stimulation inducing tetanus, 402
Toxins, 293
Tracheae, 222
in respiration, 242
Transformers, 10
Transmission, rate in earthworm, 515
Transport system, 106
Traube, 48
Treadwell, 542
Trepang, 292
Treviranus, 243
Trout, respiration, 251
Trypsin, digestion, 272
Tryptophane, composition, 276
Tubularia, regeneration, 260
Tunicates, cellulose, 30
Turtle, gas exchange, 259
rate of digestion, 333
Turtle heart, effect of salt solutions, 199
Twitch, muscle, 396, 397
Tyndall, biogenesis, 534
Typhlosole, worms, 330
Tyrosine, composition, 275

U

Ultra microscope, observations upon Brownian movement, 20
Undulating membranes, 78, 387
Unio, blood, 115
Unipolar cells, earthworm, 507
Unloading tension, 254
Urates, in excretophore cells, 432
Urea, occurrence among Echinoderms, 417
and urates, among Echinoderms, 420
Uric acid, excreted by Arachnids, 440
excreted by Hymenoptera, 440
excreted by Mollusca, 436
excreted by Protozoa, 415
Malpighian tubes, 441
occurrence among silk worms, 442

Uric acid, parasitic origin among Mollusca, 436
Urine, compared with blood plasma, 444
  freezing points, 444
Urochordata, alimentary system, 96
  circulating fluid, 100
  circulatory system, 98
  excretory system, 101
  nervous system, 102
  reproductive system, 104
  respiratory system, 99

V

Vagus, Corydalis, 523
  effect of stimulation in frog and turtle, 215
Valine, composition, 275
Valonia, permeability, 50
Valves, heart, 184
Van Beneden, circulation in Crustacea, 134
Van der Hyde, 246
Van Herwerden, 335
Van Slyke, 160, 272
  carbon dioxide in blood, 135
Vanadium, in Ascidian blood, 117
Van't Hoff, 41
  law, 251, 258
  solution, 155, 569
Vascular system, 183
  development, 178
  elastic walls, 190
  evolution, 178*ff*
  factors involved in evolution, 177
  functions of parts, 185
  incomplete, 185
Vasculo-motor apparatus, earliest type, 178
Vasculo-regulator nerves, leech, 209
Vaso-motor fibers and secretion, 290, 291
Vauquelin, 243
Veins, 184
  functions, 190
Vellellen, 325
Ventral ganglion, function in earthworm, 512
Ventriculo-bulbar junction, effect of ligature, 203
Venus, effect of nerves upon heart action, 209
Veratrin, effect on cardiac muscle, 386
  effect upon ciliary action, 386
Vernon, 258
  carbon dioxide production in tortoise heart, 215
Verworn, 61
Vibrations, range, 460, 461
Vierordt, 358
Vimtrup, 189
Vinal, respiratory system of grasshopper, 246
Virchow, 72
Visceral system, earthworm, 497
Viscosity, suspensions and suspensoids, 20
Visible spectrum, limited range, 461
Vitalism, animal behavior, 449
Vitalist, 449
Vitamins, 293, 305; A, 306; B, 306; C, 306; D, 307; E, 307
Voit, 219, 244
  and Pettenkofer, 348
Voluntary contractions, tetanus, 402
Von Fürth, 136, 244
Von Kölliker, 335
Von Lendenfeld, food taking by sponges, 321
Von Lenhossek, 506
Von Mohl, 52
Vorticella, 309
  digestion, 314
  food taking, 314

W

Wachendorff, respiration in Colpidium, 235
Warburg, 258, 260
  oxidation at fertilization of egg, 553
Washburn, osmotic pressure, 42
Wastes, elimination, 412
Water, amount in animal body, 304
  chief solvent, 13
  condition for life, 87
  content of body, regulation, 412
  cycle, 299
  dissociation, 13
  electrolyte, 13
  food, 303
  properties, 14
  respiratory medium, 224
  variations in amount ingested, 304
Water-vascular fluid, Echinodermata, 110
Webber, 335
Weber, 208
Wedensky, 474
Weinland, 232, 440
Williams, 349
Wilson, 554
Winterstein, 164
Wladimirsky, 316
Woodruff, rhythms in Paramoecium, 69
Wooley, temperature coefficient of frog's muscle, 398
Worms, digestion, 326
  excretory organs, 423
  haemoglobin, 131
  internal media, 111
  nervous system, 491*ff*
  true central nervous system, 494
Wortman, 310

X

Xanthin, absent from Echinoderm wastes, 420
  excreted by Mollusca, 437
Xanthoproteic reaction, 34

Y

Yeast fermentation, 264
Yerkes, automatic movement and irritability, 380
Yocum, 389, 452
Young, contractile vacuole of Paramoecium, 413
Yungia, Moore's experiments, 511

Z

Zooamylum, 351
Zoochlorella, 327
Zygote, 536, 545
Zymase, 264
Zymogen, 267, 294
  Protozoa, 268
  worms, 330

LANE MEDICAL LIBRARY

To avoid fine, this book should be returned on
or before the date last stamped below.

JAN 25 1952

JUN 30 1952

F31
R72
192

'F31 Rogers, C.G. 96281
R72 Textbook of compara-
1927 tive physiology.

NAME	DATE DUE
J. Luetscher	JAN 25 1952
Luetscher	JUN 20 1952
pl 7/3	